B 482880
JUL -6 1954

VOLUME 12 NUMBER 7

NUTRITION REVIEWS

JUL 21 1954

July 1954

Special Article

Early Experiences with Vitamin D in the Nutrition of Infants and Children—A Retrospect 193

Clinical Nutrition

The Determination of Human Caloric Requirements 196
Copper and Amino Acids in Wilson's Disease 198
Xanthinuria 200
Vitamin Treatment of Alcoholism 202
Nutriture and Body Composition 203

Nutrition Survey

Lunch Box Meals of French Canadian Workers 206

Nutrition and Agriculture

Environmental Factors Influencing Nutritive Value of Vegetables .. 207
Effects of Antibiotics on Plant Growth 211

Animal Nutrition

Relation of X-Disease to Vitamin A in Cattle 212
Changes in Magnesium Metabolism in Cattle 214

Experimental Nutrition

Biologic Synthesis of Ascorbic Acid 215
Organic Components of Mature Enamel 217
Vitamin B_{12} and Methyl Group Synthesis 218
The Influence of Undernutrition on Experimental Atherosclerosis ... 220
The Synthesis of Milk Fat 221

Notes

Strepogenin Activity of Synthetic Oxytocin 223
Protein Supplementation of Wheat Flour and Corn Meal by Polished Rice 223
Clinical Nutrition Briefs 224

Published Monthly at Mt. Royal and Guilford Aves., Baltimore 2, Md.

For

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK 17, N. Y.

COPYRIGHT, 1954, NUTRITION FOUNDATION INC., NEW YORK, U. S. A.

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 695 Huntington Ave., Boston, Mass.

Assistant Editor

CHRISTINE S. WILSON, 695 Huntington Ave., Boston

Associate Editors

JOSEF BROŽEK, PH.D., University of Minnesota, Stadium South Tower, Minneapolis
WILLIAM H. DAUGHADAY, M.D., School of Medicine, Washington University, St. Louis
CHARLES S. DAVIDSON, M.D., Thorndike Memorial Laboratory, Boston City Hospital
JAMES S. DINNING, PH.D., Department of Biochemistry, University of Arkansas School of Medicine, Little Rock
R. W. ENGEL, PH.D., Virginia Polytechnic Institute, Department of Biochemistry and Nutrition, Blacksburg
PAUL F. FENTON, PH.D., Department of Biology, Brown University, Providence
D. M. HEGSTED, PH.D., Department of Nutrition, Harvard School of Public Health, Boston
STANLEY M. LEVENSON, M.D., Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington
ROBERT E. OLSON, PH.D., M.D., Department of Biochemistry and Nutrition, Graduate School of Public Health, University of Pittsburgh
ROBERT E. SHANK, M.D., School of Medicine, Washington University, St. Louis
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester

Editorial Committee

REGINALD M. ATWATER, M.D., American Public Health Assn.
WALLACE R. AYKROYD, M.D., Food and Agriculture Organization
GEORGE R. COWGILL, PH.D., Yale University
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
WENDELL H. GRIFFITH, PH.D., University of California
CHARLES GLEN KING, PH.D., Nutrition Foundation
HAZEL STIEBELING, PH.D., Bureau of Human Nutrition & Home Economics
LEROY VORIS, PH.D., National Research Council
JAMES R. WILSON, M.D., American Medical Association
JOHN B. YOUMANS, M.D., Vanderbilt University

Subscriptions should be sent to The Nutrition Foundation, Inc., Chrysler Building, New York 17, N. Y. Rate $4.00 per year in the United States and Canada; $4.50 in other countries.
Changes of address should be sent to The Nutrition Foundation.
Application for entry as second-class matter pending at the Post Office at Baltimore, Md.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved.

NUTRITION REVIEWS

VOL. 12 JULY 1954 No. 7

EARLY EXPERIENCES WITH VITAMIN D IN THE NUTRITION OF INFANTS AND CHILDREN—A RETROSPECT

My introduction to vitamin D metabolism came before its identity was known. I came to the State University of Iowa in 1920 to work with Dr. Amy L. Daniels on quantitative studies in infant nutrition. Slowly and at times painfully, we taught ourselves the technics necessary for successful collection of excreta of infants without exhausting the infant from too much immobility. After determining the possible effect of heat treatment on utilization of minerals from cow's milk, Dr. Daniels went on to learn the reasons back of a startling earlier finding. She had been feeding a partly skimmed milk formula, and was concerned lest the babies not get enough "fat-soluble A," so had added to the feedings a little cod liver oil, known to be rich in this vitamin. The babies had thrived amazingly well, conspicuously better than those fed a high cream mixture by Dr. A. H. Byfield, then head of Pediatrics, and the metabolic studies were begun in order to find out the reason for this rapid growth.

Then the news broke in 1922 that cod liver oil contained two vitamins; the second, which was named D, was the rickets-preventive factor. The babies being studied at Iowa had not only escaped rickets, but had grown very well and were very sturdy babies. Vitamin D did more than just prevent disease.

Looking back from this distance, it is obvious that, in addition to the excellent reasoning and careful study that lay back of these sound observations on Dr. Daniels' part, the element of luck entered in, as it often does. It was pure luck indeed that the cod liver oil used was potent, for Dr. Byfield felt certain that the efficacy of cod liver oil was in direct proportion to its unpalatability. The oil came in gallon tin cans, like varnish cans, so was not exposed to light, but it was never refrigerated.

With one analyst, and using gravimetric methods for calcium, collection of data was slow. The study did not get published until 1929, by which time it included data on other forms of vitamin D.

In 1924, Dr. Jeans came to Iowa and was an interested observer of these early studies. The same year, Hess and Steenbock independently discovered that many foods, like children, could develop vitamin D when given ultraviolet irradiation. Soon, everything from milk to sausage was being irradiated to provide the marvelous new vitamin. Attempts were made to assay vitamin D preparations. Chemical methods proved impractical and various methods were brought forward for assay by means of curative effect of vitamin D on rickets in young rats. Several standard units were in vogue, and dosage could not be readily converted from one unit to another. Many studies reported in the literature referred simply to "cod liver oil," or "irradiated milk" (irradiated by the investigator), neither of which was standardized. "Yeast milk," obtained by feeding irradiated yeast to cows, had about the same potency for rats as irradiated milk. It was uneconomical, as the unitage in milk was about that per pound of cow, but it avoided "tampering" with milk, a legal question in some states.

The confusion of unitage and efficacy which began in the late twenties continued into the thirties. The first studies had been published with no knowledge of unitage; in later studies, the unitage might be given in "rat units" which differed from one investigator to another. As an example of the existing confusion, Alfred Hess, whose publications in the field of infantile rickets

were impressive, published a series of articles stating that one rat unit of cod liver oil was equal to four of viosterol (irradiated ergosterol) for the infant, that yeast milk was more effective for the baby than cod liver oil, on a rat unit basis, and that irradiated milk was superior to all others, unit for rat unit of vitamin D.

In the late twenties, after time out for a PH.D. at Michigan, I returned to Iowa. Dr. Jeans and I were interested in the other burning question of the moment in the field of infant feeding. Was the curd or the buffer the important factor in causing the excellent utilization of Marriott's acid milk formulas? The introduction of evaporated milk for infant feeding also needed study of its usefulness. One teaspoon of cod liver oil daily had been sufficient to make the babies studied by Dr. Daniels thrive—why use more? In all of these studies, one teaspoonful of cod liver oil was given daily to each baby. Fortunately, during these years, we used a cod liver oil from the same firm, standardized through the careful work of Dr. Bills. The milk studies showed all methods of curding equally effective for the baby, so by the early thirties the accumulated data were useful also as control studies for comparison of types of vitamin D. In 1934, the international unit for vitamin D came into use, and with it the hope that the confusion could soon be resolved.

By now, the infant Committee on Foods of the American Medical Association was becoming involved in the question of efficacies of the various forms of vitamin D. They had succeeded in curbing the injudicious addition of vitamin D to foods, and had limited the Seal of Acceptance to vitamin D milk. But how to deal with these claims for the efficacy of the various milks was a real problem.

Vitamin D milk had caught the public fancy. Viosterol, a concentrate of irradiated ergosterol (D_2) in an oily medium, appeared on the market and also enjoyed wide popularity. Mothers were impressed with the fact that their babies no longer smelled like fish nor was their clothing stained yellow from spilled fish oil.

Still the relative merits of the different forms and dispersions of vitamin D for the human infant were unsolved. To add to this confusion of dosage, roentgenographic diagnosis of rickets became ultrarefined, so that no two investigators seemed to have the same criteria for diagnosing rickets. The incidence of this nutritional disease was estimated all the way from 15 to 65 or more per cent of the infant population of a given area. We, like many others, turned our attention to unraveling the question of the efficacy of types (and dispersion) of vitamin D. We studied the effectiveness of the vitamin for retention of calcium and phosphorus and for linear growth of well infants closely housed and under complete control. Others studied efficacy of rickets prevention and rate of linear growth in larger outpatient groups. Interpretation of rickets prevention was difficult because of the different criteria used to determine presence or absence of rickets. In 1936, Dr. Jeans was asked by the Committee on Foods of the American Medical Association to review the whole subject critically. With the quantitative data from our own laboratory, he was able to show that certain slight but well-defined changes at the epiphyses appear in the roentgenograms of infants receiving little or no vitamin D, are not seen in roentgenograms of infants receiving moderate amounts of vitamin D, about 100 I.U. daily, but appear again in infants given higher amounts of the vitamin and showing more rapid growth. Thus, interpretation of such roentgenographic changes cannot be made without more knowledge of the infant than can be gained from the roentgenogram alone.

With this confusion of interpretation cleared, enough studies of standardized vitamin D milks, oils and concentrates were available to permit the conclusions that all vitamin D from animal sources had the same

value for infants, unit for unit, whether it was given as fish liver oil, as the unsaponifiable matter from fish liver oil in a medium dispersible in milk, or as irradiated milk. This review also concluded that milk containing 135 I.U. of vitamin D to the quart would prevent rickets in infants when ingested in customary quantities by infants, but that this amount was close to the minimum requirements, whereas milk containing 400 I.U. of vitamin D to the quart allowed a good margin of safety.

By 1937, the Committee on Foods had become the American Medical Association Council on Foods, and extended its previous decisions to indicate that while vitamin D "of vegetable origin" (D_2) might not be equal in efficiency to vitamin D_3, for the infant, the differences were too slight to be significant in the prevention of rickets, retention of calcium and phosphorus, or linear growth. For these studies, each of the two types of vitamin D had been compared at two levels of intake, in milks containing 135 I.U. or 400 I.U. per quart. The question of concentrated versus dispersed sources began to occupy the spotlight.

The dictum of the Council on Foods that vendors of 400-unit vitamin D milk were permitted greater advertising claims than those selling 135-I.U. vitamin D milk sounded the knell for irradiated milks. Off-flavors developed when milk was irradiated to this high a potency. The expense of irradiation equipment and the easement of state laws, which now permitted the addition of vitamin D concentrates to milk, also hastened the change-over. Soon all vitamin D milk contained 400 I.U. per quart.

The decision of the Evaporated Milk Association to add 400 units of vitamin D per reconstituted quart to all evaporated milk without increase in price proved a significant public health measure. Evaporated milk was the cheapest dependable source of milk for infant feeding. Its fortification automatically protected about three fourths of the artificially fed infants in the United States against rickets.

In the late thirties agreement became general that, on a unitage basis, vitamin D concentrates in oily mediums were not so effective for the infant as the dispersed forms. Pharmaceutical firms heightened the search for dispersing agents which would make the fat-soluble vitamins miscible with water.

Also, in the thirties, along with the studies on infants, Dr. Jeans and I managed to study the calcium and phosphorus retentions of children from 1 to 12 years old. We found that, in each group, some children used these nutrients efficiently without added vitamin D while others needed the vitamin to ensure good retention. We could not tell from the appearance of any child which group he belonged to. The amount of the vitamin suitable for infants, 300 to 400 units daily, seemed to permit adequate retention in the less efficient children and to do no harm to the efficient ones, so we advocated the ingestion of that amount of vitamin D daily during the entire growth period, even though active rickets is rare past two years of age.

Thus, the majority of the questions concerning requirement of various types of vitamin D during human growth were satisfactorily solved within two decades. The discovery of vitamin D, the later discoveries that there were several vitamins D, two of which were of practical importance, and the finding of a practical test for potency, the "line test," all occurred in the decade of the twenties.

The early years of the thirties were marked by rapid progress in the chemistry, physiology and requirement of vitamins D for various species, including man. The reckless irradiation of foods for the sake of advertising propaganda was brought under control by the new Committee on Foods of the American Medical Association. These years also were marked by confusion of unitage, of diagnosis of rickets, and of ideas

concerning relative efficacy of the various forms of vitamin D. In 1934, the international unit for vitamin D was adopted. By 1937, the questions of efficacy of vitamins D_2 and D_3 were settled, and by 1939 there was general agreement that vitamin D dispersible in watery mediums was more readily available to the infant than the vitamin in a concentrated oily source. The controversies of the thirties were over. By the close of the next decade the chief concern had become overdosage with fat-soluble vitamins highly concentrated and in dispersible form. We had run full gamut.

GENEVIEVE STEARNS
Research Professor of Pediatrics
State University of Iowa
Iowa City

THE DETERMINATION OF HUMAN CALORIC REQUIREMENTS

The emphasis on specific nutrients during the past quarter century has given to many the impression that the only important dietary ingredients are the vitamins. There has been a tendency to ignore or relegate to a minor role the caloric content of the diet and the caloric demands of the individual. J. B. Orr and I. Leitch (*Nutrition Abstr. Rev.* **7,** *509 (1938)*) attempted to re-emphasize the importance of calories by saying that "however important the requirement for specific nutrients may be, the energy requirement is primary and, under ordinary conditions, will be satisfied before, and even at the expense of any other."

Earlier in this century, calories loomed so high on the nutrition horizon that Graham Lusk in his book, "The Science of Nutrition" (*3d edition, W. B. Saunders Co., Philadelphia (1917)*), discussed calorimetry in the introduction and described the Atwater-Rosa respiration calorimeter in the first chapter and starvation in the second chapter. The large chambers used in the early studies of energy metabolism have either been dismantled or are currently used only on rare occasions. The cost of the chamber and its high operating expenses have combined to limit its use.

E. Kofrányi and H. F. Michaelis (*Arbeitsphysiol.* **11,** *148 (1940)*) described a light-weight gas meter which may help to extend the studies on caloric requirements for different types of activity. (To date, this apparatus is almost unknown in the United States.) The apparatus, which is carried on the subject's back, measures the volume of expired air and collects a sample which is a constant aliquot (0.2 to 0.7 per cent—the exact fraction can be varied for each study) of the expired air. A series of expired air samples can be collected by changing the rubber sampling chamber (a football bladder). By this means, the time interval for a study can be extended to a number of hours when a mask is used (G. Lehmann, E. A. Müller, and H. Spitzer, *Ibid.* **14,** *166 (1950)*); when a mouthpiece is used in place of the mask for the collection of expired air, some subjects develop a dry mouth after fifteen minutes which interferes with proper breathing (D. Orsini and R. Passmore, *J. Physiol.* **115,** *95 (1951)*). The oxygen and carbon dioxide contents of the sample of expired air and of the atmosphere are determined by analysis in an apparatus such as the Haldane. From the consumption of oxygen and production of carbon dioxide the energy expenditure can be calculated. Essentially, the experimental setup is the same as that in which the Douglas bag has been used. The primary advantage of the Kofrányi and Michaelis apparatus over the Douglas bag is that it permits the collection of gas over a fairly long period of time without imposing any undue restriction upon the subject.

Orsini and Passmore (*loc. cit.*) found that both the Kofrányi and Michaelis apparatus and the Douglas bag gave similar results for

short periods of standardized laboratory work. They then used the Kofrányi and Michaelis apparatus for the study of energy expenditure required to walk up and down stairs. The apparatus was very satisfactory for such studies. An interesting auxiliary observation was that walking downstairs required more energy than horizontal walking. (Many people assume that going down steps requires practically no energy above that incident to standing.) The extra energy, according to them, was spent in maintaining posture while stepping down the stairs.

The Kofrányi and Michaelis apparatus has been used extensively in Germany for studies on the energy cost of a large variety of household tasks (W. Droese, E. Kofrányi, H. Kraut, and L. Wildeman, *Arbeitsphysiol.* **14,** *63 (1949)*) and industrial operations (Lehmann *et al., loc. cit.*; *Nutrition Reviews* **9,** *273 (1951)*). The former report shows the differences in energy that 3 women expended in doing the same type of household work. The report on the caloric requirements of industrial workers was carried out on men who had been accustomed to the work for a long time and thus provides a valuable report of energy output for a host of jobs as done by modern mechanized methods.

The problems and difficulties in any extended caloric balance study are shown by the work of R. Passmore, J. G. Thomson, and G. M. Warnock (*Brit. J. Nutrition* **6,** *253 (1952)*). Five young men lived in the laboratory for thirteen days, during which time their food intake was measured and analyzed for protein, fat and carbohydrate. From these results the caloric intakes were calculated. The caloric expenditure for each task engaged in by each subject was assessed by means of the Kofrányi and Michaelis apparatus. From the length of time spent at each task and the energy expenditure, the caloric output of each subject was calculated. A five-day period of strenuous exercise was inserted in the middle of the experiment. During this time, the subjects were in negative caloric balance. All of the caloric deficit was restored during the subsequent four days. The caloric expenditure by 3 of the subjects for the thirteen-day period was about 5 per cent less than the dietary intake. For these men the weight increases ranged from 0.14 to 0.34 kg. One of the subjects consumed 11 per cent more calories than he expended; his weight gain was 2.7 kg.; the other consumed 8 per cent less calories than he expended; he lost 2.5 kg. There was no constant relation between the caloric excess (or deficit) and the body weight gain (or loss). There were obviously changes in hydration associated with the body weight alterations.

The change in the composition of the body will always pose a problem for all caloric balance studies until such time as very small changes in the content of protein and fat can be detected in the intact organism. Lacking such methods, the only alternatives are to make the study under such conditions that changes in body composition are minimized, or to make a complete balance which would require a measure of the carbon lost in the urine, feces and expired air, together with the caloric intake. With proper precautions, it would appear that experiments of this type might be carried out with the Kofrányi and Michaelis apparatus.

In the study by Passmore *et al.* (*loc. cit.*), the subjects should have been kept in the laboratory until body weight equilibrium had been achieved—it was suggested that part of the body weight gain was a compliment to the cuisine. They should have been trained for the hard work before the experiment started, in order to avoid the improvement in work performance which comes with training.

The work of Passmore and co-workers suggests that a caloric balance study is possible using the Kofrányi and Michaelis apparatus. There is as yet no exact indication of the accuracy that can be achieved

with this apparatus. Should it be as accurate as it appears, then it will be of considerable aid in resolving some of the wide discrepancies currently existing in caloric allowances promulgated by different organizations. The establishment of reliable caloric requirements will assume increasing importance if a large-scale rationing program becomes necessary due to any future emergency. The importance attached to calories in such a program was emphasized by the late Sir Jack Drummond when he said, "Time and time again during the war years striking illustrations were recorded of the harmful effects of caloric deficiency on working capacity, physical condition, and morale" (*Pub. Health Reports* **67,** *857 (1952)*).

COPPER AND AMINO ACIDS IN WILSON'S DISEASE

Recent studies of the metabolic defect found in Wilson's disease have been concerned with the possible role of copper and of abnormal amino acid metabolism. This syndrome, described as a disease entity by Wilson in 1912, is a progressive disease of early life and is frequently familial in character. Pathologically it is characterized by degeneration of certain regions of the brain, especially the corpus striatum, and by cirrhosis of the liver. Clinically, muscular rigidity and tremor are associated with manifestations of liver disease.

Numerous studies in the past have produced evidence that the disease is inherited in a recessive manner. This evidence has been based upon recorded case histories and, therefore, may have been subject to error if sufficient details were not recorded. A more definitive investigation has been carried out by A. G. Bearn (*Am. J. Med.* **15,** *442 (1953)*), who studied the genetic histories of fourteen families in which at least one member had Wilson's disease. In the 26 patients from these families he was able to demonstrate a high first-cousin consanguinity rate (37.5 per cent) and accordingly he has suggested that the disease is inherited in an autosomal recessive manner. Calculations of the genetic ratio strongly supported this hypothesis.

A. Rumpel (*Deutsch. Z. f. Nervenheilk.* **49,** *54 (1913)*) reported increased copper deposits in the kidney and liver of patients with Wilson's disease. F. Haurowitz (*Z. f. physiol. chem.* **190,** *72 (1930)*) found increased copper content in both brain and liver. This work was later substantiated by B. M. Mandelbrote, M. W. Stanier, R. H. S. Thompson, and M. N. Thruston (*Brain* **71,** *212 (1948)*) and by J. N. Cumings (*Ibid.* **71,** *410 (1948)*). The latter investigator also demonstrated increased amounts of copper in the urine. The effect of BAL (2–3 dimercaptopropanol) on the copper excretion of patients with Wilson's disease was demonstrated by Mandelbrote, who found increased excretion of copper in the urine following administration of this agent. This was substantiated by D. Denny-Brown and H. Porter (*New Engl. J. Med.* **245,** *917 (1951)*) and by Cumings (*Brain* **74,** *10 (1951)*).

Temporary remission of signs and symptoms and considerable amelioration of the disease were observed in some patients receiving BAL. Although A. J. Glazebrook (*Edinburgh Med. J.* **52,** *83 (1945)*) reported elevated serum copper concentrations in Wilson's disease, other investigators (J. N. Cumings, *Brain* **71,** *410 (1948)*; Bearn, *loc. cit.*) have not confirmed this, and have found either normal or decreased serum copper levels.

The role of ceruloplasmin, a blue globulin found in serum which is involved in the transport mechanism of copper, has been investigated by I. H. Scheinberg and D. Gitlin (*Science* **116,** *484 (1952)*). In normal individuals all of the copper found in serum is bound to this globulin. These investigators demonstrated that the ceruloplasmin level

was low in Wilson's disease and that it did not account for all the serum copper present. They advanced the theory that, in this disease, as in hemophilia, there is deficiency of a specific protein.

Bearn (*loc. cit.*) similarly demonstrated a low ceruloplasmin level and subsequently Bearn and H. G. Kunkel (*Proc. Soc. Exp. Biol. Med.* **85,** *44* (*1954*)) investigated the localization of Cu^{64} in serum fractions following oral administration. They found in normal subjects and patients with cirrhosis of the liver that there was an immediate uptake of copper following administration of Cu^{64} by a serum albumin fraction. The radioactivity then shifted within a few hours to the alpha-2 globulin (ceruloplasmin), the major copper-containing protein in human serum. When Cu^{64} was administered to patients with Wilson's disease, however, the radioactivity was confined to the albumin peak and little or no localization of copper in the alpha-2 globulin fraction occurred.

The demonstration of an abnormal excretion of amino acids in this disease was described by L. Uzman and D. Denny-Brown (*Am. J. Med. Sci.* **215,** *599* (*1948*)), who found a persistent and often massive aminoaciduria. This finding has been amply substantiated by other investigators. This excessive amount of amino acids in the urine occurred not only in patients with the disease, since C.-H. de Verdier (*Acta med. Scandinav.* **138,** *344* (*1950*)), B. Hood and S.-E. Fagerberg (*Ibid.* **140,** *374* (*1951*)) and Uzman and Hood (*Am. J. Med. Sci.* **223,** *392* (*1952*)) made the same finding in asymptomatic siblings of a family affected with the disease.

A. M. Cooper, R. C. Eckhardt, W. W. Faloon, and C. S. Davidson (*J. Clin. Invest.* **29,** *265* (*1950*)) found that the aminoaciduria was not related to an elevated blood amino acid level as measured by plasma alpha amino nitrogen, nor did there appear to be any defect in amino acid metabolism as measured by the way the patients handled glycine and glutamic acid. There was no specific pattern of the excessive amino acid excretion and the aminoaciduria did not appear to be related to the severity of the liver disease. In addition, there was no increased percentage loss of amino acids following the ingestion of protein or the infusion of amino acids. These workers postulated that the aminoaciduria could be explained by a unique type of lowered renal threshold for amino acids in these patients, since the finding that infusions of amino acid did not further increase the degree of aminoaciduria is inconsistent with the usual concept of a threshold mechanism.

W. B. Matthews, M. D. Milne, and M. Bell (*Quart. J. Med.* **21,** *425* (*1952*)) showed that the urinary excretion of copper in 2 patients with Wilson's disease could be increased by the ingestion of large doses of glycine and alanine. It was inferred that copper was probably excreted in the form of a copper-chelate in which the amino acid forms a nonionizable complex with copper. Uzman and Hood (*loc. cit.*) demonstrated that in addition to aminoaciduria present in asymptomatic members of a family with Wilson's disease, abnormal amounts of peptides were also excreted. Uzman further demonstrated (*Am. J. Med. Sci.* **226,** *645* (*1953*)) that the peptides also appeared in the urine of patients with Wilson's disease, but, in this instance, were bound in a chelate bond with copper. He showed that the bulk of the urinary copper excreted by patients with Wilson's disease was in this form. The daily output of copper bore no relationship to the severity of the aminoaciduria.

The above-described findings provide some insight into the possible defects involved in the etiology of hepatolenticular degeneration. These observations illustrate the familial nature of the defect in amino acid metabolism, which appears to precede the development of both the hepatic and the lenticular degeneration. The chronic nutritional drain resulting from the persistent aminoaciduria may well explain the development of hepatic cirrhosis (H. P.

Himsworth, "Lectures on the Liver and Its Diseases," *2d edition, pp. 98–101, Harvard University Press, Cambridge (1950)*; Uzman and Hood, *loc. cit.*; I. B. Brick, *New Engl. J. Med.* **247,** *635 (1952)*). This concept would also be in keeping with the well known observation that families of patients with Wilson's disease also have a high incidence of cirrhosis of the liver.

Despite the attraction of the above theoretic mechanism for the hepatic manifestations, the chronic loss of amino acids does not explain the lenticular degeneration. Although it has been demonstrated that there are increased amounts of copper in the brains of patients with Wilson's disease and that BAL may ameliorate rigidity and tremor in these patients, there is much evidence against the possibility that abnormal copper metabolism is the primary defect in the disease. According to Uzman (*loc. cit.*) and Uzman and Hood (*loc. cit.*), aminoaciduria and peptiduria have been demonstrated to occur in asymptomatic patients and in asymptomatic siblings of patients long before the development of lenticular degeneration. At the same time, copper could not be demonstrated in the urine of these patients. Likewise, although BAL will ameliorate symptoms in some patients, this often is an inconstant and temporary occurrence, and the disease progresses in its usual course (Brick, *loc. cit.*).

The metabolic defect which gives rise to the lenticular degeneration has not as yet been demonstrated. The finding of associated aminoaciduria and peptiduria and the demonstration that the greatest amount of copper in the urine is bound in a chelate bond to the peptides suggests that the peptide copper chelate is the form in which copper passes from the plasma into the glomerular filtrate. This also raises the possibility that the excessive urinary excretion of the metal is a result of the competition for tubular reabsorption between the peptide complex and the amino acids, both of which are present in high concentration in the tubular fluid. Uzman (*loc. cit.*) postulates that there may be a defect in the enzyme system of brain tissue which allows the accumulation of peptides locally. Since copper readily forms a bond with peptides, local deposition could then be expected to occur.

Another possible mechanism for increased copper deposition may be postulated on the basis of the work of Scheinberg and Gitlin (*loc. cit.*) and Bearn and Kunkel (*loc. cit.*). Due to the low ceruloplasmin level in Wilson's disease, serum copper is largely bound to an albumin fraction. That this is a weak bond can probably be deduced from the fact that normally copper is quickly transferred from the albumin fraction to a globulin fraction (ceruloplasmin), in which form it is transported. Copper, being loosely bound to serum albumin, may thus be released readily into tissue and urine as a copper-peptide complex. However, this hypothesis lacks considerable evidence.

In summary, at least two metabolic defects in Wilson's disease have been demonstrated which may be interrelated. The inability to transport copper normally in the blood due to the low ceruloplasmin level and the excessive excretion of both amino acids and peptides appear to be well established phenomena which are probably genetically transmitted. The heavy copper deposition and the abnormally high copper excretion would appear to be secondary to these basic defects. Whether or not these two basic defects can in turn be traced to a common denominator in blood, brain, liver, or kidney remains to be elucidated.

XANTHINURIA

Urinary xanthine stones, described in 1817, have been reported in only about two dozen patients. A disorder in purine metabolism has been postulated, but its nature is

not established. C. E. Dent and G. R. Philpot (*Lancet* **I,** *182 (1954)*) have recently had the opportunity to study a young child with xanthinuria and a xanthine calculus.

A 4½ year old girl, previously in good health, was admitted to the hospital because of urinary frequency and hematuria. Physical examination was negative. No excess calcium, cystine, or other amino acids were found in her urine which was sterile, contained numerous red blood cells, a few white blood cells and a trace of protein. No opaque calculi were found on roentgenologic examination. A week after admission the child passed a stone in her urine, after which her urinary symptoms rapidly improved.

The calculus was nonopaque to x-rays, and on analysis by a chromatographic technic proved to be principally xanthine. About 5 per cent of the stone by weight was protein. Chromatographic analysis of the patient's urine revealed the presence of considerable xanthine, but no uric acid. A small amount of urinary uric acid (about 100 mg. per gram of creatinine) appeared to be present by chemical determination. The urinary xanthine was markedly abnormal, about 600 mg. of xanthine per gram of creatinine being excreted. This is fifteen to twenty times the amount normally present.

Plasma concentrations of both uric acid and xanthine, determined chromatographically, were very low, less than 1 mg. per 100 ml. of plasma being found. Plasma uric acid measured chemically was about 0.5 mg. per cent. Plasma xanthine was not measured chemically because of lack of a suitable method.

The authors then examined the patient's family, which included her sister, father, mother, 5 aunts, 2 surviving uncles and 3 surviving grandparents. All were healthy. None gave any history suggestive of renal stones; all excreted normal quantities of uric acid; none excreted xanthine.

Dent and Philpot suggested two possibilities for the presence of xanthine and relative absence of uric acid in the urine in this patient. First, the patient may have been born with a lack of xanthine oxidase which is normally present in the liver and presumed to be essential for the conversion of xanthine to uric acid. If this were the case, and the constancy of the biochemical defect is established, Dent and Philpot point out that the condition will fit the criteria of an "inborn error of metabolism." A second possibility is that owing to a defect in kidney function, xanthine is excreted so rapidly that there is not time for its conversion to uric acid. In keeping with this possibility is the finding of small amounts of uric acid in plasma and the urine as measured by chemical, but not chromatographic, means. If the second possibility is true, then this patient would fit into the category described by Dent as a "deviation of metabolism." Dent and Philpot mention that uric acid excretion by Dalmatian coach hounds and cystine excretion in cystinuria appear to fit in this category.

The authors point out certain differences between the patient studied by them and some of the patients with xanthine stones previously reported by other investigators. In one of these latter patients the blood uric acid level was reported as normal (C. K. Pearlman, *J. Urol.* **64,** *799 (1950)*), and in another a "uric acid stone" was reported to have been present in the renal pelvis of the kidney opposite to that in which the xanthine stone was found (J. Israel, "Chirurgische Klinik der Nierenkrankheiten," *Berlin (1901)*). They also cite a patient with low blood uric acid, but normal uric acid excretion (E. Praetorius and J. E. Kirk (*J. Lab. Clin. Med.* **35,** *865 (1950)*).

Dent and Philpot point out that patients with xanthinuria are apt to have xanthine stones because of the marked insolubility of xanthine, particularly in acid urine. They suggest, as prophylactic measures, that such patients take alkalis by mouth and have a high water intake.

Further investigations are of course needed to establish the fundamental nature

of the disturbances in purine metabolism noted. Estimation of hepatic xanthine oxidase might be accomplished by analysis of tissue obtained by liver biopsy. As Dent and Philpot indicate, the clinical application of chromatographic technics may result in finding considerably more patients with disorders in purine metabolism.

VITAMIN TREATMENT OF ALCOHOLISM

In the last decade strains of rats have been developed which under controlled conditions consume larger amounts of alcohol than other strains (R. J. Williams, *Quart. J. Studies Alcohol* **7,** *567 (1947)*). Williams has postulated that an inherited metabolic pattern may be the factor responsible for the special appetite for alcohol in such animals. Indeed, as a result of his experimental studies, Williams has introduced a general theory of "genetotrophic disease," in which the underlying lesion is a "partial" block of genetic origin in the metabolic machinery of a given animal (Williams, E. Beerstecher, and L. J. Berry, *Lancet* **I,** *287 (1950)*; *Nutrition Reviews* **8,** *257 (1950)*).

In applying the genetotrophic theory to alcoholism in man, Williams has hypothesized that the abnormal craving of the alcoholic for alcohol may result from a metabolic disorder (perhaps in the metabolism of alcohol) somewhat as the craving for salt in a patient with Addison's disease results from a derangement in salt and water metabolism.

Since a variety of vitamins participate as prosthetic groups in coenzymes concerned with the breakdown of alcohol to carbon dioxide and water, vitamin supplements have been administered to experimental animals (R. A. Brady and W. W. Westerfeld, *Quart. J. Studies Alcohol* **7,** *499 (1947)*) and to human subjects (J. A. Smith, P. A. Dardin, and W. T. Brown, *Ibid.* **12,** *381 (1951)*) in an attempt to determine whether, by a "mass action" effect, the postulated partial block can be overcome and the pattern of alcoholic intake modified.

Although studies in animals indicated that the voluntary intake of alcohol by rats may be modified by administration of the B-complex vitamins (Brady and Westerfeld, *loc. cit.*), preliminary studies in man were inconclusive because of the small number of patients studied, the relative brevity of the experiments, and lack of controls (Smith *et al., loc. cit.*).

A more extensive project involving observations on the effect of multiple vitamin therapy in the treatment of alcoholism in man has been reported (M. F. Trulson, R. Fleming, and F. J. Stare, *J. Am. Med. Assn.* **155,** *114 (1954)*). Ascorbic acid, vitamins A and E, and the B-complex vitamins including vitamin B_{12}, folacin, choline and inositol, were prescribed each day for alcoholic patients in amounts usually associated with the therapy of severe deficiency states. For example, the dose of thiamine employed after the second week of treatment was 30 mg. per day. The ascorbic acid intake was 500 mg. per day. Capsules containing inert material and which resembled the vitamin capsules were used as placebos in the control studies. A total of 207 patients with alcoholism were observed over a two-year period. Of this group, 87 still were being followed after two years of the clinic program.

The patients were placed in four categories with respect to their therapeutic response: total abstinence, controlled drinking, "improvement" and, lastly, no change in drinking pattern. First, the authors considered the patients under study as a whole, without distinguishing between those treated for a relatively short time (one to three months) and those under treatment for more than twelve months. It was determined that 16 per cent of the vitamin-treated group

became totally abstinent, while 21 per cent of the group receiving placebos stopped drinking completely. Seventeen per cent of the patients on vitamin therapy reported a change to controlled drinking while only 2 per cent of the patients on placebos displayed such a change. Only a small number of patients receiving vitamins were included in the "improvement" category and approximately 70 per cent of the patients given vitamin therapy displayed "no change" in their drinking habits.

Of 23 patients who were switched from placebos to vitamins after three or more months, 11 appeared to improve to a varying degree; of 14 patients who were switched from vitamins to placebos after a similar period, only 2 improved.

In attempting to appraise their findings, the authors point out that no reliable method was available for determining the number of patients who faithfully took the prescribed capsules and pills. Indeed, it was learned by careful interviews that many of the subjects were remiss in taking their medications. It was also difficult to evaluate with precision the degree of improvement of the patients since such information usually came from the patients themselves, and alcoholic patients are notoriously unreliable. In addition to their vitamin therapy the patients also were seen regularly by a physician and a nutritionist, and from time to time were given other medications such as amphetamine, mephenesin, and disulfiram (Antabuse). Because of the many variables involved, it was believed desirable to prolong the studies to a point where temporary effects produced by a brief trial with drug therapy or psychotherapy would be minimized.

Accordingly, a group of 25 patients who had been taking vitamin medication for thirteen to twenty-four months were considered separately. Of this group who had been under prolonged vitamin treatment 16 patients displayed some degree of improvement, compared to the one out of 7 who had improved during the same time interval while taking the placebo.

As a result of their two years experience with this kind of therapy, the authors conclude that vitamins are not generally effective in the treatment of alcoholism. However, the cases which maintained improvement while under vitamin therapy are of considerable interest. It seems pertinent to inquire whether switching the 16 improved patients to placebos after their long period of improvement might have resulted in a return to uncontrolled alcoholism. In the light of the apparent beneficial effect of vitamins in *some* cases, it is worth considering whether alcoholism (like obesity) might have a multiple etiology, including a "metabolic" variety.

It is to be hoped that the interesting studies described by Trulson and her associates will be continued until the role of vitamin therapy in the treatment of alcoholism can be determined with greater finality. Possibly, too, as a result of such studies, further light will be shed on the applicability of the genetotrophic theory to alcoholism and, by extension, to other clinical problems.

NUTRITURE AND BODY COMPOSITION

The evaluation of morphologic aspects of nutritional status (nutriture) has been the subject of two extensive papers published from the Laboratory of Physiological Hygiene, University of Minnesota. One deals with a narrower segment of the problem, the role of anthropometry in the assessment of nutriture (J. Brozek, *Am. J. Phys. Anthropol.* **11,** *147 (1953)*), while the other surveys the whole field, with special reference to a quantitative estimation of body fat in the adult man (A. Keys and

Brozek, *Physiol. Rev.* **33,** *245 (1953)*). The latter paper is as much a review of the literature, citing some 300 references, as an original contribution to the methodology, especially as far as the densitometric analysis of body composition is concerned. In this area important modifications of the system developed by A. R. Behnke and his collaborators were proposed (*see Nutrition Reviews* **9,** *265 (1951)*; **10,** *181 (1952)*; **11,** *165, 301 (1953)*).

The growing appreciation of the potential usefulness of anthropometry as well as the awareness of the need for rethinking the approach and for standardization of the measurements is reflected in the statements of the Joint FAO/WHO Expert Committee on Nutrition (Report on the Second Session, *WHO Technical Report Series No. 44, 63 (1951)*): "The physical dimensions of the body are, to a considerable degree, dependent on its nutrition. Anthropometric measurements are therefore of importance in assessing existing nutritional status and in obtaining information about past nutritional history. Measurements of body weight in relation to height are widely used in assessing nutritional status in relation to health, and many anthropometric measurements and indices are applied and advocated for this purpose. Other kinds of measurements, particularly of thickness of the skinfolds, deserve greater attention as a criterion of relative fatness and 'calorie status.' There are, however, no generally accepted norms for the evaluation of such measurements nor, indeed, generally accepted standard methods of making the measurements themselves."

The paper on "Measuring Nutriture" (Brozek, *loc. cit.*) was presented as the opening address in a panel discussion organized within the framework of the annual meeting of the American Association of Physical Anthropologists. The other prepared papers presented at the panel dealt with the measurement of obesity in chronic disease control program (J. W. Rion, *Am. J. Phys. Anthropol.* **11,** *258 (1953)*), the reliability of serial measurements (W. M. Krogman, *Ibid., p. 259*), subcutaneous fat and body form of women (B. Škerlj *et al., Ibid.*), changes in body fat during a six-week tour of duty in the Arizona desert (P. T. Baker, *Ibid., p. 260*), and measurement of body volume, and thus of body density, by air displacement (R. J. Wedgwood and R. W. Newman, *Ibid.*).

In Brozek's paper an attempt was made to portray both the *positive* role of body measurements (and of the newer methods for the description of body composition in terms of tissue masses) in the evaluation of human nutriture and the *limitations* of nutritional anthropometry. It was recognized that nutritional status is a complex concept in which clinical symptoms, laboratory assessment of the levels of essential nutrients in bodily tissues and fluids, as well as body dimensions and body composition must be considered. Consequently, the use of any single datum such as "anthropometric index of nutritional status" grossly simplifies and at times distorts the over-all picture. Severe nutritional deficiencies, such as scurvy, are found at times in the individuals who appear well nourished and pellagra may develop in the presence of obesity. On the other hand, good health is compatible with a wide range of values for height and weight. The fact that child growth and the structure of the adult human body are influenced by genetic factors and activity as well as by nutrition must be kept in mind.

Nevertheless the mass and the composition of the body represent a fundamental criterion of nutriture. These characteristics may be evaluated on the basis of anthropometric data, including measurements of the principal body tissues accessible externally (bones, muscles, subcutaneous adipose tissue), measurements made from x-ray photographs, and the newer biophysical and biochemical methods for the breakdown of the gross body weight into

fat and the fat-free body mass and its cellular, mineral and fluid components.

The methods were critically discussed by Keys and Brozek (*loc. cit.*), with special reference to the estimation of body fat in adult man. In addition to the impressionistic appraisal of a patient by the clinician, comparison of actual weight with a "standard" weight is most widely used as a criterion of nutritional status. The fact that the anatomic constituents of body weight (skin, adipose tissue, muscles and internal organs, extracellular fluid, skeleton) may vary in their relative contribution constitutes the fundamental limitation of body weight. In the tabular standards usually only height, age, and sex are taken into account. A better standard is obtained by considering also the width of the skeletal framework.

The subcutaneous adipose tissue constitutes a large fraction of the fat deposited in the body. Consequently, the measurement of the thickness of the layer of skin plus the subcutaneous tissue should be useful as a criterion of relative fatness. Pinching a fold of "skin" has been a time-honored procedure in selecting live turkeys for market quality as well as in the physician's evaluation of the patient's nutriture. Absence of standardized instruments, with known and constant pressure at the contact surfaces of the skinfold calipers, and of agreement on sites have been obstacles for a wider utilization of this technic. At present, developmental research is going on in several laboratories, both in the United States and in England, which should close the existing gaps in our information about the effect of caliper pressure, consistency of repeated measurements, and the best "weights" in adding several skinfolds.

A novel method was proposed for the estimation of body fat from body density, defined as the ratio of body mass to body volume. The body of normal young men (age 25 years, standard weight) was used as the standard of reference. The density of such a body was determined empirically (*cf.* J. Brozek, *Fed. Proc.* **11,** *784 (1952)*) as 1.063 g. per cubic centimeter. When men gained weight through simple overeating, the average density of this gain was 0.948 and, consequently, their body density became lower. It was estimated that the gain consisted of about 62 per cent fat (equivalent to ether extract), 24 per cent cellular matter, and 14 per cent extracellular fluid. On the basis of these data the equation $\Delta F = (5.427/D) - 5.106$ was developed in which D is body density and ΔF indicates the amount of fat, expressed as proportion of the total body weight, by which the given individual differs from the standard. Assuming that the body of the reference man contains 14 per cent of fat, the total fat content of an individual with the density D may be calculated from the equation $F = (4.201/D) - 3.817$.

These equations are valid only under the assumption of normal hydration of the body. However, the deviations from the normal values can be assessed by determining the volume and mass of the extracellular fluid, and the effect on body density is allowed for in the equation for estimating body fat.

Assuming, as has been done by several authors, that the body mass (M = 1) can be separated into fat (F) and a fat-free portion (1-F), and that the concentration of water (A) in the fat-free body mass is constant ($A = c(1 - F)$), body fat as a proportion of the total body weight may be calculated from the equation $F = 1 - (A/c)$.

As in the densitometric method the prediction equation is made more precise if the effect of differences in extracellular hydration is eliminated. Starting with a theoretic breakdown of the body mass into fat, total body water, cell solids and bone mineral, Keys and Brozek end up with the equation: $F = 1 - 1.563A + 0.563E$ for the calculation of body fat (F) based on the determination of total body water (A) and

the extracellular fluid (E). All symbols represent fractions of the body weight.

Age, physical activity and body build were discussed as sources of variation in body composition. From the point of view of the nutritionist the application of the methods to the study of gains and losses of body weight is of particular interest. It is estimated that one kilogram of gross weight gain represents a tissue mass having a value of about 6000 calories. However, this value and the underlying composition of the tissues lost or gained are "constant" only in a relatively narrow range of weight changes around the "normal" body weight, especially as weight losses are concerned. With more severe degrees of calorie deficit the loss of "active" (nonfat, mostly muscle) tissues becomes progressively a larger percentage of the weight loss. In extreme starvation the fat depots almost completely disappear and the weight changes are accounted for by the cells of muscles and internal organs, together with changes in hydration. The amount of water in the body may decrease, as in "dry" cachexia, or may increase, as is seen in some famine victims with severe edema.

The composition of the human body is an important facet of the nutritional status (nutriture). While in the laboratory and the clinic the densitometric approach and the determination of fluid compartments are the methods of preference, in field work improved anthropometric methods will provide important new tools. Here further developments should proceed in several directions: (1) improvement of methods for the evaluation of man's physique from photographs, (2) estimation of anatomically defined tissue masses from external and radiologic measurements, and (3) development of equations for predicting total body fat from skinfolds, using the values obtained from density and total body water as criteria.

LUNCH BOX MEALS OF FRENCH CANADIAN WORKERS

Lunch box meals are a relatively recent institution in French Canada, both because of prevailing social habits and because this region has only lately become heavily industrialized. For this reason it appeared of interest to check on the composition and nutritional value of the foods chosen by Canadian workers for consumption at their place of work.

In a careful and extensive study A. Gasnier, M. B. Loranger, and L. Brouha (*Rev. canad. de biol.* **12,** *355 (1953)*) studied 2668 lunch box meals brought at different seasons of the year by the workers of the Aluminum Company of Canada, Ltd., at Shawinigan Falls, Quebec. Results were analyzed in terms of frequency of appearance of various food groups according to the classification of G. H. Berryman and C. Chatfield (*J. Nutrition* **25,** *23 (1943)*) which comprises 17 food groups. Nutrient contents were calculated using the table of Berryman and Chatfield and the Lunch Food Composition Table of L. Randoin, P. Le Gallic, and J. Causeret ("Tables de composition des aliments," Institut Scientifique d'Hygiène Alimentaire, *Lanore, Paris (1947)*).

From the standpoint of qualitative description, Gasnier and his co-workers found first of all that the composition of meals in terms of foodstuffs varied very little with the shift; that is, with the time of day at which they were to be consumed. Bread was the universal basis of the meal. Only one worker had substituted cereals for bread. Butter and pastry were almost as universally consumed. Meat and fish products were less common. Only one meal out of two contained one such source of animal proteins. Fruit was rarely consumed, but green vegetables and raw tomatoes were

commonly eaten during the part of the year when they were cheap in the local markets. Tea was the favorite drink, with milk a distant second. Not a single worker consumed either sweet or carbonated beverages. One worker brought fruit juice. The briefness of the lunch period (twenty minutes) did not seem to affect the meal pattern, as workers of another section who were allowed one hour for their lunch brought similar meals.

It is of interest to note that these lunch box meals reflect to a large extent the dietary habits encountered among French Canadian families of the less prosperous classes (M. V. Ball, L. Brouha, A. Gasnier, and J.-L. Tremblay (*Laval méd.* **11,** *1025 (1946)*).

In quantitative terms it was found that, as expected, the size of the meals varied considerably from one man to the next and for the same man, depending on the season. On the average, the meals contained 170 g. of carbohydrates, 50 g. of fat, 32 g. of protein and a total caloric value of 1220 calories. The fat content of the meal was thus relatively high, as it represented more than one third of the calories. Butter was the main source of fat by far. The greatest part of the protein was of vegetable origin, being contributed for the main part by cereals. The average calcium content was of the order of 200 mg. and showed a particularly wide range of variation (from 75 mg. to over 1 g.). The Ca:P ratio was of the order of 0.45. Other interesting averages were iron, 5 mg.; vitamin A, 2000 units; thiamine, 0.6 mg.; riboflavin, 0.45 mg.; niacin, 5 mg.; ascorbic acid, 25 mg.

The authors comment on the following points: high fat content of the diet, low ratio of animal proteins to vegetable proteins, low calcium-to-phosphorus ratio, low niacin and low riboflavin. It is of interest to note that in their studies of the nutritional status of school children of the Gaspé (Ball *et. al., loc. cit.*), Gasnier and Brouha had noted the high frequency of subclinical signs of riboflavin and niacin deficiencies.

The authors suggest a number of relatively inexpensive additions to the standard lunch box meal which would considerably improve its nutritional value: replacement of the commonly consumed pastry by cheese and milk-containing desserts, greater use of celery and green vegetables. If economically feasible, a greater consumption of citrus fruits and meat products would also be beneficial.

The Province of Quebec is evolving more rapidly than is any other large part of North America. It will be of interest to see whether in the coming years French Canadian food habits will also be substantially modified.

ENVIRONMENTAL FACTORS INFLUENCING NUTRITIVE VALUE OF VEGETABLES

The emphasis of group effort in food and nutrition research resulted in the establishment of the Southern Cooperative Group some ten years ago. Cooperative research effort was established by Agricultural Experiment Stations in Arkansas, Georgia, Louisiana, Mississippi, North Carolina, Oklahoma, Puerto Rico and Texas, with the cooperation of the U. S. Plant, Soil, and Nutrition Laboratory, U. S. Department of Agriculture at Ithaca, New York, and the Weather Bureau, U. S. Department of Agriculture. Broadly, the research effort has been directed at the influence of location, variations in soil, and weather on the nutritive value of vegetables.

In the course of these investigations, many studies were devoted to methods of taking field samples, handling, subsampling, and analytic technics. The results of methodology studies were published as *Southern Cooperative Series Bulletin No. 10,*

March 1951, by all of the cooperating Experiment Stations under the title, "Studies of Sampling Techniques and Chemical Analyses of Vegetables."

In the ten-year period from 1943 to 1953, the Southern Cooperative Group has published 10 bulletins dealing with various environmental influences on the nutrient composition of selected vegetables with respect to calcium, phosphorus, iron, ascorbic acid, carotene, thiamine and riboflavin.

The effect of fertilizer and environment on the iron content of turnip greens was investigated at 19 different locations in Georgia, Mississippi, Oklahoma, Virginia, South Carolina and Texas (*Southern Cooperative Series Bulletin No. 2, April 1944*). Great variations were found in the iron content of this vegetable with different climatic conditions and soil types. The high value was obtained at Blairsville, Georgia (0.72 mg. of iron per gram dry matter), and the low at West Point, Mississippi (0.10 mg. per gram). The most uniform effect of fertilizer treatment was a decrease in iron content caused by nitrogen application which was observed in 26 of 29 experiments. Although the size of the decrease varied with place, this fertilizer effect was highly significant in all groups of experiments and averaged 0.051 mg. of iron per gram of dry matter when the mean iron content for all treatments was 0.255 mg. per gram dry matter. Thus, the influence on iron content of conditions associated with location or place of growth were much greater than the fertilizer effects.

Season seemed to influence the nitrogen effect since this effect was observed to be significantly smaller in 10 fall crops than in 18 spring crops. In 6 of 7 experiments, the average iron content was significantly lower in the fall crop than in the spring crop. No relation was found between total rainfall during the growing season and the iron content of turnip greens. The nitrogen fertilizer effect appeared to be greater when the soil was high in organic matter than when it was low.

The calcium and phosphorus content of turnip greens was also lowered with soil nitrogen fertilization. The decrease was generally smaller for calcium than for iron and much smaller and more irregular for phosphorus (O. A. Sheets *et al.*, *J. Agr. Res.* **68,** *145 (1944)*).

An effect of real practical importance was the increase in yield which resulted from nitrogen fertilization. To obtain a good yield of turnip greens on the majority of southern soils, it is necessary to apply nitrogen fertilizer. Despite the negative effects of nitrogen on iron content, the use of nitrogen fertilizer is thus not contraindicated. Location appears to influence the iron content of this vegetable significantly and were the crop grown under soil conditions that resulted in low iron content, it would contribute only about 20 to 25 per cent as much iron to the human diet as turnip greens grown under soil conditions that resulted in high iron content.

The effect of fertilizer and environment on the calcium, phosphorus and iron content of cowpeas was investigated in Georgia, Mississippi, Oklahoma, South Carolina and Virginia (*Southern Cooperative Series Bulletin No. 4, November 1946*). The mean calcium and phosphorus contents of 736 samples of cowpeas from 23 experiments (13 locations) were 0.078 and 0.5019 per cent, respectively (dry matter basis), and the mean iron content of 576 samples from 18 experiments was 0.065. Only one significant correlation was found between the constituents studied, a negative correlation between iron and calcium contents.

Again, as in the turnip green studies, the mineral content of cowpeas was influenced to a much greater extent by location than by fertilizer treatment. Location means for phosphorus varied from 0.372 per cent to 0.647 per cent, those for calcium from 0.640 per cent to 0.123 per cent, and those for iron from 0.040 to 0.078 per cent.

The only correlation between environmental factors characterizing location and mineral content of cowpeas was a highly significant reduction in phosphorus associated with increase in soil organic matter.

The composition of southern peas (cowpeas) was reported in *Southern Cooperative Series Bulletin No. 15, November 1951.* Dry matter, protein, fat, starch, total sugars, reducing sugars, sucrose, nitrogen-free extract, dextrins, crude fiber, ash, ascorbic acid, carotene, thiamine, riboflavin, calcium, phosphorus and iron were determined in samples at one or more locations. A total of 28 varieties of cowpeas was tested at three growth stages (snap pod, green and dry peas).

On the dry weight basis the three classifications of samples differed a little in their average contents of protein, phosphorus and thiamine. The largest difference was in fat content, which was higher in green peas. Snap pods (stage of growth in which pods and peas can be prepared together as a dish) had a higher mean content of fat, sugar, dextrins, crude fiber, ash, calcium, iron and riboflavin, and a lower average content of starch and nitrogen-free extract, than green or dry peas. Snap pods also averaged much higher in ascorbic acid and carotene than green or dry peas.

Varieties differed substantially in protein and thiamine, and the contents for these nutrients were positively correlated. Differences in composition associated with geographic location were statistically significant. Large differences between locations were found for riboflavin. For example, snap pods grown in Georgia averaged 0.92 mg. per 100 g. (dry weight basis) contrasted with an average of 2.52 the same year in Mississippi.

Studies on the lima bean (*Southern Cooperative Series Bulletin No. 5, May 1946*) involved the cooperative efforts of Georgia, Mississippi, South Carolina, Virginia and the U. S. Regional Vegetable Breeding Laboratory at Charleston, South Carolina. The effects of cooking, storage, bean maturity, location and variety were tested at 6 locations. Large differences were observed in the ascorbic acid, thiamine and riboflavin contents of lima beans grown at different locations. For ascorbic acid, the high value of 120 mg. per 100 g. (dry weight basis) was obtained at Experiment, Georgia, while the low value of 65 mg. per 100 g. was observed at Charleston, South Carolina. Respective thiamine and riboflavin values ranged from lows and highs of 653 and 1000 (thiamine), and 193 and 387 (riboflavin) mg. per 100 g. of lima beans.

Age of the plant did not affect the concentration of these nutrients as long as maturity of the beans remained constant. Maturity was, however, of major importance. Ascorbic acid, thiamine and riboflavin contents decreased with maturity, but the decrease was greater with ascorbic acid and riboflavin than with thiamine.

In order to obtain highest nutritive value with respect to these vitamins this study recommends that the lima beans be harvested at the earliest marketable stage of maturity; that the product be cooked as soon after harvest as possible and for as short a time as possible; that storage should be in the pod and that the beans should not be shelled until ready for processing or cooking.

Further studies on the lima bean were reported in the *Southern Cooperative Series Bulletin No. 12, June 1951.* In these experiments it was established that the ascorbic acid content of lima beans was higher in late plantings than in early plantings at some locations. Neither the thiamine nor riboflavin content was affected by planting date. Location at which the crop was grown again appeared to influence vitamin content of lima beans more than planting date.

The influence of fertilizer and environment on the ascorbic acid content of turnip greens was investigated cooperatively by R. Reder, L. Ascham, and M. S. Eheart at

Oklahoma, Georgia and Virginia Experiment Stations (*J. Agr. Res.* **66,** *375 (1943)*). Again, location appeared to have a major influence. The mean ascorbic acid content of greens at Norfolk, Virginia (2.41 mg. per gram, dry weight basis), was nearly twice that of greens at Blacksburg, Virginia (1.28 mg. per gram). In all experiments the influence of location was 13.75 times as great as the most important average effect of fertilizer treatment (decrease in ascorbic acid content with potassium fertilization).

The highest ascorbic acid content of 3 spring crops (1.91 mg. per gram) was found where average daily rainfall was lowest and 49 per cent of the growing season was under clear sky conditions. The lowest average ascorbic acid content (1.28 mg. per gram) occurred at a location of heaviest rainfall and least amount of sunshine.

The effect of maturity, nitrogen fertilization, storage and cooking on the ascorbic acid content of turnip greens was reported in *Southern Cooperative Series Bulletin No. 1, 1943*. The 2 varieties studied were significantly different in average ascorbic acid content (Shogain averaged 129.6 and Seven Top averaged 146 mg. per 100 g., fresh basis). The former variety had a statistically significant higher moisture content than the latter so that on a dry basis it was superior in ascorbic acid content. Nitrogen fertilization was without influence on the ascorbic acid content of turnip greens. There was a highly positive correlation between rainfall and ascorbic acid content, in contrast to the results reported in the earlier study on turnip greens.

Investigations on the carotene and ascorbic acid content of sweet potatoes have been reported in *Bulletins 3* and *30*, dated Dec. 1945 and Oct. 1953, in the Southern Cooperative Series. The most uniform effect of fertilizer was a lowering of the moisture and ascorbic acid contents of sweet potatoes with combined nitrogen and phosphorus fertilization and with combined phosphorus and potassium fertilization. Wide variations between varieties were found in the carotene content of sweet potatoes; the extremes ranged from 0.5 to 44.6 mg. per 100 g. (dry basis). Varieties outstandingly high were Allgold and Goldrush. Both of the widely grown varieties, Unit I Porto Rico and Bunch Porto Rico, were below the average of the 22 varieties or lines tested with respect to carotene content. Mean yields of marketable potatoes for high carotene varieties compared favorably with yields of the widely grown varieties relatively low in carotene.

Location effects were again important. Thus, the carotene content of 5 varieties grown at Blacksburg, Virginia, was consistently lower (17 mg. per 100 g.) than when these varieties were grown at Baton Rouge, Louisiana (31 mg. per 100 g.). The reverse was true for ascorbic acid. At Blacksburg, 5 varieties averaged 119 mg. of ascorbic acid per 100 g. (dry weight basis), and at Baton Rouge, the average was 70 mg.

As in the earlier studies, fertilizer effects on the vitamin content of vegetable matter appear to represent at most only a small part of the total environmental effect on the plant. The very marked location effects point to other environmental factors that must be of major importance in influencing the vitamin and mineral composition of vegetables.

The series of publications that has come out of the Southern Cooperative Group experiments represents a concerted effort to define more clearly some of the environmental factors that influence plant composition. The vegetable crops that have received attention in these studies (sweet potatoes, cowpeas, lima beans and turnip greens) were selected for study because they are widely grown in the southern states and represent a major contribution to the vitamin and mineral content of diets in this region.

EFFECTS OF ANTIBIOTICS ON PLANT GROWTH

Antibiotics are now used extensively in animal feeds, and it appears that their most important function in animal growth stimulation is associated with their ability to control a low disease level that would have an adverse influence on growth rate.

Since the beginning of the "antibiotic era" in animal nutrition, the role of antibiotics in plant nutrition has been under investigation. Generally, the results have been negative, perhaps because the tests were usually conducted with antibiotic levels in excess of 50 parts per million (p.p.m.) in the growth medium. In much of the earlier work the antibiotic preparations used were impure and were carriers for potent plant hormones (M. Being, R. Signer, and W. H. Schopfer, *Experientia* **3,** *291 (1947)*).

Studies of the possible mechanism of action of antibiotics in plant growth were recently reported (L. G. Nickell and A. C. Finlay, *J. Agr. Food Chem.* **2,** *178 (1954)*). These workers chose a duckweed (*Lemna minor*) as the test plant. This plant grows readily on a chemically defined liquid medium. The medium used consisted of salts (K, N, Mg, Ca, sulphate, chloride, B, Mn, Zn, Cu, Mo and Fe), sucrose, and 3 vitamins (thiamine, pyridoxine and niacinamide). Growth during test periods was measured in a culture room at a temperature of 22.5° C. The results were expressed in terms of wet weight attained. A group of antibiotics was screened under these conditions at concentrations of 1, 5, 10 and 20 p.p.m. in the medium and for a three-week growth period. Great variations were noted in the effects of the antibiotics on plant growth. The antibiotics that consistently stimulated growth were bacitracin and penicillin G. In both cases the stimulation increased with concentration of the antibiotic in the growth medium. For example, with penicillin G at 5 p.p.m. an increase in growth of 80 per cent over the control was observed, whereas at 20 p.p.m. this increase was 330 per cent. Antibiotics that inhibited growth were aureothricin, catenulin, neomycin and subtilin.

Oxytetracycline stimulated slightly at low concentrations but was inhibitory at higher concentrations. The stimulatory effect of this antibiotic was increased when the growth period was extended to eight weeks. At a 5 p.p.m. concentration, a 350 per cent increase in growth was observed, whereas no stimulation occurred when the concentration was increased to 20 p.p.m. The increase in stimulation from oxytetracycline with increase in incubation time suggested that the stimulation might be due to breakdown products of the drug rather than to the drug itself. This point of view was confirmed in auxiliary studies. Experiments were also conducted to determine whether or not the presence of living plant tissue had a stimulatory effect on the degradation of oxytetracycline, and the results indicated that the presence of living plant tissue had little or no effect on degradation of the antibiotic into growth stimulatory products. A number of available degradation products of oxytetracycline were tested in separate experiments, but the results gave no clue as to the structural entity essential for plant growth stimulation.

Some evidence was obtained to indicate that plants grown under control conditions (no antibiotic) secrete toxins or growth inhibitors into the medium and in this way check their own growth. The role that antibiotics play in the detoxification of such toxic products was not determined. It seems doubtful, however, that the use of antibiotics could be practical in agriculture through such a mechanism since leaching, diffusion, bacterial decomposition, adsorption by soil, and other factors would prob-

ably prevent excessive accumulation of toxic products secreted by plants grown under natural conditions.

These studies have offered no direct clue to the mechanism of stimulatory action of antibiotics in the nutrition of animals.

There are, of course, many other hurdles that must be cleared before the results obtained under these carefully controlled conditions can be interpreted in terms of field conditions. Clay soils, for example, are known to adsorb many antibiotics with a high degree of irreversibility (P. Siminoff and D. Gottlieb, *Phytopathol.* **41,** *420 (1951)*; N. Martin and Gottlieb, *Ibid.* **42,** *294 (1952)*).

The present work, as well as earlier work by L. G. Nickell (*Antibiotics and Chemotherapy* **3,** *449 (1953)*; *Proc. Soc. Exp. Biol. Med.* **80,** *615 (1952)*), in which plant growth stimulation was exhibited with antibiotics, rules out the indirect effect of these compounds through control of microbial population since all the experiments were conducted under aseptic conditions. The action of the antibiotics in plant growth stimulation therefore does not seem to fit the hypothesis that has been widely proposed for animals.

Some possibilities considered by Nickell and Finlay were: (1) primary activity could be represented by degradation products of the antibiotic; (2) properties other than antibacterial are responsible, *e.g.*, chelation; (3) toxic plant metabolites are neutralized; (4) cell membrane permeability is affected; or (5) hormone metabolism of the plant is modified.

The observation reported by the same authors that the potent antitubercular compound, isonicotinic acid hydrazide, was plant-growth stimulatory under their conditions obviously raises the question as to whether the growth stimulatory effects observed should be considered at all unique for antibiotics alone.

RELATION OF X-DISEASE TO VITAMIN A IN CATTLE

Bovine hyperkeratosis (X-disease) is a condition characterized by the thickening, folding, hardening and wrinkling of the skin, particularly over the neck and shoulders of affected animals. Although sporadic outbreaks of this disease in cattle have been observed for a number of years, when the highly chlorinated naphthalenes were identified as causative agents of the disease, it was soon established that the disease as produced experimentally simulated many of the features of vitamin A deficiency. A rapid decline in blood vitamin A levels is routinely observed in affected animals (R. W. Engel and W. B. Bell, *Nutrition Reviews* **11,** *97 (1953)*). Recent studies conducted at the University of Wisconsin indicate that the hyperkeratotic symptoms typical of X-disease in cattle can be produced by the topical application of oil-based insecticide carriers (W. G. Hoekstra, R. J. Dicke, and P. H. Phillips, *Am. J. Vet. Res.* **15,** *47 (1954)*).

Twelve bull calves 2 to 6 months of age were divided into 6 lots and subjected to the following treatments: lot 1, control; lot 2, sprayed with 0.05 per cent of lindane in mineral seal oil; lot 3, sprayed with 0.5 of methoxychlor in mineral seal oil; lot 4, sprayed with 3.2 per cent of lethane and 2.3 per cent of thanite (thioxyanate compounds) in mineral seal oil; lot 5, X-disease controls receiving orally 0.1 g. of highly chlorinated naphthalene in corn oil per day for six days; and lot 6, sprayed with mineral seal oil only. The oil-based insecticides and oil were applied to the calves and the walls of the pens with hand sprayers.

All calves that were subjected to spraying with the oil or oil-based materials (lots 2, 3, 4, and 6) developed wrinkling of the skin on the neck and shoulders within a

week after treatment was initiated and this later developed into the extremely thickened and hardened skin that is characteristic of bovine hyperkeratosis. The younger animals appeared to be more severely affected than the older calves.

Blood vitamin A determinations were conducted at weekly intervals. A consistent drop in blood vitamin A occurred within a week following the first application of the oil-based insecticides or the oil alone. In general this was represented by declines from control levels of about 12 micrograms of vitamin A per 100 ml. of plasma to values of about one-half this amount. Although these declines in blood vitamin A were less severe than those in the calves of lot 5 that received highly chlorinated naphthalene orally, the results established rather clearly that the development of skin lesions from topical application of the oil spray were associated with an apparent decrease in circulating vitamin A.

The mineral oil used in the Wisconsin experiments is a petroleum fraction with no additives, commonly used as an insecticide carrier for stock sprays. The dosage of the spray used in the experiment (about 17 ml. per calf daily) was approximately two times the recommended safe level as advocated by the manufacturer.

Further studies relating the bovine hyperkeratotic condition to vitamin A deficiency have been reported by these investigators (W. G. Hoekstra, R. E. Hall, and P. H. Phillips, *Am. J. Vet. Res.* **15,** *41* (*1954*)). Young calves were fed a pelleted hyperkeratosis-producing feed and died with terminal pneumonia without developing the typical hyperkeratotic syndrome. The same feed, when given to older calves, resulted in typical skin changes of hyperkeratosis. The hyperkeratotic syndrome was partially alleviated with vitamin A therapy (600,000 to 750,000 I.U. per calf over a nine- to twelve-day period). In general, these results indicated that animals suffering from the hyperkeratotic syndrome as a result of toxic pellet consumption responded with a prospective favorable prognosis if the blood vitamin A values returned to normal during treatment and did not decline following termination of treatment. Cases in which vitamin A therapy failed to return blood vitamin A levels to normal terminated fatally.

Hoekstra and co-workers concluded that vitamin A plasma levels are valuable in the diagnosis of hyperkeratosis and in following the progress of the disease under treatment. Their work again emphasizes the apparent high antivitamin-A potency of bovine hyperkeratosis-producing agents. Some doubt is cast on the earlier suggestion that this antivitamin-A effect is an interference with the conversion of carotene to vitamin A. In the Wisconsin experiments it was noted that vitamin A in the plasma was depressed by increased intakes of the hyperkeratotic agents while vitamin A itself was being administered.

Other blood constituents studied by the Wisconsin workers were carotene, ascorbic acid, niacin, vitamin B_6, vitamin B_{12}, sodium and potassium. None of these was significantly altered during the course of the disease. In this connection recent preliminary observations by E. L. Hove (*J. Nutrition* **51,** *609* (*1953*)) deserve mention. Hove noted that in cattle, presumably poisoned by highly chlorinated naphthalenes and exhibiting chronic symptoms of X-disease, there was a decrease not only in vitamin A but also in the vitamin E concentration in the blood plasma. His results support the general thesis that lipid-soluble toxic chemicals (carbon tetrachloride, pyridine, tri-o-cresol phosphate, and chlorinated naphthalenes) act through a pro-oxidant effect on unsaturated lipids and fat-soluble vitamins.

Although the mode of action of the agents responsible for hyperkeratosis in cattle remains to be determined, it is becoming

increasingly evident that the nutritional status of the animal with respect to vitamin A, and apparently also vitamin E, is of vital importance.

CHANGES IN MAGNESIUM METABOLISM IN CATTLE

One aspect of an intensive grassland agriculture is the heavy use of artificial fertilizers for the maintenance of high-yielding sward. In the British Isles, where grassland agriculture is practiced much more extensively than in this country (*Nutrition Reviews* **10,** *339 (1952)*), experiments are under way to determine whether the extensive use of artificial fertilizers can be related to a disease in cattle known as grass staggers or grass tetany. This condition is associated with sudden decreases in the serum magnesium level, symptoms of hyperesthesia (excessive sensibility), and convulsive tetany. Low serum calcium levels are also often seen in the late stages of the disease, particularly among animals that are near the point of death.

The occurrence of hypomagnesemia with or without clinical symptoms is most generally seen in animals shortly after they are turned to grass after stall-feeding on conventional rations (grain, hay, and silage). The fact that the pastures usually supply more magnesium than the ordinary stall-fed rations suggests that grass tetany represents a physiologic dysfunction rather than a simple magnesium deficiency. It has been demonstrated that the feeding of magnesium oxide or magnesium-rich mineral supplements is beneficial in preventing the rapid fall in serum magnesium that occurs when animals are suddenly changed from stall-feeding to grass (I. J. Cunningham, *New Zealand J. Sci. Technol.* **15,** *414 (1934)*; W. M. Allcroft and H. H. Green, *J. Comp. Path.* **51,** *176 (1938)*; Allcroft, *Vet. J.* **103,** *75 (1947)*).

A recent paper by some British workers describes preliminary studies in which it was demonstrated that specific fertilizer applications to pastures could influence the incidence of hypomagnesemia and grass tetany in dairy cows (S. Bartlett *et al.*, *Brit. Vet. J.* **110,** *3 (1954)*).

In 1952 two pasture plots were included in the study. Plot A received an application of magnesium oxide (2500 pounds per acre) in February and sulfate of ammonia applications in March and April (300 pounds per acre each month). Plot B received applications of sulfate of ammonia only (300 pounds per acre) in February and March.

Three of the 4 cows grazed on plot A did not develop hypomagnesemia after twenty days of grazing. In the fourth cow the serum magnesium levels fell from 2.7 to 0.5 mg. per cent in the first week, but this animal failed to develop symptoms of grass tetany. Of 8 cows turned onto plot B, 5 developed severe hypomagnesemia (0.5 to 0.6 mg. of magnesium per 100 ml. of serum), and 4 of these exhibited typical symptoms of grass tetany. Mild hypomagnesemia was noted in 2 of the 3 remaining animals.

Four additional cows were pastured on plot B, but received a supplementary feed (concentrate fed at the rate of 1 pound per 4 pounds of milk produced). Although moderate declines in serum magnesium occurred, none of the cows developed symptoms of grass tetany. It was further observed that when the sward was converted from a mixed grass-clover stand to a predominantly clover stand by heavy application of potash, less severe drops in serum magnesium occurred in grazing cattle.

In the 1953 experiment the trends noted in the previous season were confirmed. On plot A the serum magnesium levels in 4 of 5 cows remained within the normal physiologic range. The fifth cow experienced a

mild hypomagnesemia that returned to normal in ten days. After three to four weeks of grazing these cows were transferred to plot B. All animals experienced hypomagnesemia in three days. Six of 7 cows initially grazed on plot B (no magnesium fertilizer) experienced a drop in serum magnesium levels. Two of these died in seven and seventeen days, respectively, after grazing was initiated. The third developed tetany symptoms. The remaining 4 cows were transferred to plot A (magnesium-fertilized in 1952). The 3 which had experienced hypomagnesemia returned to normal in six days.

With one exception, in all cases where physical symptoms of grass tetany were observed in these experiments the blood serum calcium levels had declined from the normal range of 8.0 to 10.9 mg. per cent to the range 6.8 to 8.0 mg. per cent.

The authors point out that their experiments do not contribute direct evidence regarding the fundamental cause of hypomagnesemia in grazing animals. On the assumption that the consumption was 30 pounds of herbage (dry matter) per cow daily, they calculated that the magnesium-fertilized plot yielded a daily magnesium intake per cow of about 34 g. They also calculated that plots not fertilized with magnesium resulted in intakes of about 20 g. It was felt, however, that this difference in magnesium intake could not serve as the only explanation for the beneficial results obtained with magnesium fertilization of the soil. In other experiments herbage supplying as little as 9 to 11 g. of magnesium per cow daily was only rarely observed to produce hypomagnesemia. One possibility they considered was that soil treatment with magnesium resulted in a changed soil condition such that the production of "tetany" agents was suppressed.

Whatever may be the explanation for their results, the evidence presented by the British workers suggests strongly that fertilization of grassland soil with magnesium can have real effects on blood magnesium levels of grazing animals.

BIOLOGIC SYNTHESIS OF ASCORBIC ACID

The structural similarity of L-ascorbic acid and certain hexose sugars has often stimulated speculation as to the possible role of carbohydrates as precursors of the vitamin. S. S. Jackel, E. H. Mosbach, J. J. Burns, and C. G. King (*J. Biol. Chem.* **186,** *569 (1950)*) administered glucose labeled in all positions with C^{14} to chloretone-treated rats and isolated ascorbic acid uniformly labeled with C^{14}. This strongly suggested the possibility of a direct conversion of the 6-carbon sugar into the 6-carbon ascorbic acid without prior rupture of the carbon chain. F. A. Isherwood, Y. T. Chen, and L. W. Mapson (*Biochem. J.* **56,** *1 (1954)*) have approached this problem by setting up several possible schemes for the synthesis of L-ascorbic acid. The validity of each scheme was tested by administering one or more of the postulated intermediates to cress seedlings or to rats and measuring the resulting production, if any, of ascorbic acid.

The dual test system employed here is of particular interest. The cress seeds were placed on a piece of fine nylon cloth in a porcelain jar. This cloth in turn rested on a piece of filter paper. The intermediate to be tested was dissolved in water and introduced into the jar in amounts sufficient to just wet the seeds. Use of nylon cloth permitted the frequent changes of nutrient solution made necessary by the slow decomposition of some of the materials tested. Control seeds were moistened only with water. Germination was allowed to proceed

in the dark at a temperature of 20° C. The seedlings introduced into solution of possible intermediates were tested to show the penetration of these compounds into the cell sap. Both control and experimental seedlings were tested for their content of ascorbic acid. A given compound was assumed to be a possible intermediate in ascorbic acid synthesis if the ascorbic acid content of the experimental seedlings exceeded that of the controls germinating in plain water.

Ascorbic acid synthesis in rats was tested by maintaining the animals on a semi-synthetic diet until the urinary excretion of the vitamin became relatively constant. At this time 100 mg. of the possible intermediates were injected subcutaneously and urinary ascorbic acid was determined quantitatively. A compound was judged to be a possible intermediate if its administration led to a significant increase in the excretion of ascorbic acid. The authors recognized the possibility that a given compound might stimulate ascorbic acid excretion without being itself a precursor of the vitamin.

The compounds tested included glyceraldehyde (to test the possibility of chain-cleavage and recondensation), various sugars, the gamma-lactones of a long series of sugar acids and the methyl ester of galacturonic acid. Gamma-lactones of the sugar acids and the methyl ester of galacturonic acid were employed because they were known to penetrate the cell membrane more readily than the free acids.

Since the gamma-lactones of D-glucuronic acid and L-gulonic acid stimulated the synthesis of ascorbic acid by cress seedlings, the authors felt justified in postulating the conversion of D-glucose into D-glucuronic acid by oxidation, followed by reduction to L-gulonic acid (with inversion of the numbering of the carbon chain). This in turn might be followed by oxidation to L-ascorbic acid. Similar experiments on the possible intermediates in the conversion of D-galactose to L-ascorbic acid showed that D-galacturonic acid methyl ester and L-galactono-gamma-lactone appreciably increased the synthesis of L-ascorbic acid by cress seedlings and by rats. Presumably D-galactose may be oxidized to galacturonic acid, this in turn reduced to L-galactonic acid (inversion of carbon numbering), and the latter oxidized to L-ascorbic acid. About 3 per cent of the administered lactone appeared in the urine as ascorbic acid.

The conversion of D-glucose and D-galactose into L-ascorbic acid by the two pathways suggested by the authors is in agreement with the findings of H. H. Horowitz, A. P. Doerschuk, and C. G. King (*J. Biol. Chem.* **199,** *193* (*1952*)) that D-glucose labeled with C^{14} at the 1 position led to the urinary excretion in chloretone-treated rats of ascorbic acid labeled in the 6 position. It also agrees with the report of Horowitz and King (*Ibid.* **200,** *125* (*1953*)) that administration of 6-labeled glucose gave rise to 1-labeled ascorbic acid. The direct conversion of labeled glucose (carbons 1 or 6) into labeled glucuronic acid has been shown by J. F. Douglas and King (*Ibid.* **202,** *865* (*1953*)). More recently Horowitz and King (*Ibid.* **205,** *815* (*1953*); *Nutrition Reviews* **12,** *155* (*1954*)) showed the transformation of uniformly labeled glucuronolactone into uniformly labeled ascorbic acid in rats.

In a second paper in this series, F. A. Isherwood, Y. T. Chen, and L. W. Mapson (*Biochem. J.* **56,** *15* (*1954*)) report on the investigation in some detail of the relationship of D-glyceric acid to the synthesis of L-ascorbic acid in cress seedlings. Chromatographic analysis of the cell sap of cress seedlings showed that among the several organic acids present, only D-glyceric acid changed in concentration in proportion to the concentration of L-ascorbic acid. Glyceric acid was actually isolated and identified. The relationship between D-glyceric acid and L-ascorbic acid was shown to be pH-dependent, increasing pH leading to increasing ratios of glyceric to ascorbic acid. When the seedlings were provided with materials which could influence the rate of

ascorbic acid synthesis, several interesting results were obtained. Supplying ascorbic acid in the nutrient medium increased the seedlings' content of the vitamin while depressing the glyceric acid content. Similarly, the gamma-lactones of D-glucuronic acid and L-gulonic acid increased the seedlings' content of ascorbic acid while depressing the glyceric acid concentration.

The possibility that D-glyceric acid might be a precursor of L-ascorbic acid was ruled out by the finding that the introduction of glycerate into the nutrient medium increased the glyceric acid content of the cell sap, but did not significantly affect the formation of ascorbic acid. Similarly, it was shown that in the chloretone-treated rat the excretion of L-ascorbic acid, as well as D-glyceric acid, increased over that observed in the untreated animals. Administration of D-glyceric acid to such rats did not alter the urinary output of L-ascorbic acid. Thus there appears to be some relation between the synthesis of glyceric acid and ascorbic acid, but it is clear that glyceric acid is not a direct precursor of the vitamin.

In the third paper of the series, L. W. Mapson, F. A. Isherwood, and Y. T. Chen (*Biochem. J.* **56,** *21* (*1954*)) report on their studies on the synthesis of L-ascorbic acid by plant mitochrondria. Pea seedlings were employed as sources of mitochondria because their ascorbic acid oxidase content is relatively low during the early hours following germination. As expected from the two preceding papers of the series, L-galactono-gamma-lactone was found to serve as precursor in the synthesis of ascorbic acid by the mitochondrial preparations. Contrary to expectations, however, L-gulono-gamma-lactone was not converted to the vitamin by the mitochondria, although excised pea embryos grown in this possible precursor gave rise to ascorbic acid. The ability of embryos to convert gulonic acid lactone into ascorbic acid suggests strongly that the synthetic pathway from glucose via glucuronic acid and gulonic acid is open to the plant, but the necessary enzyme systems have been inactivated during the isolation of the mitochondria, or else that the conversion by this pathway involves oxidations other than those mediated by mitochondria.

ORGANIC COMPONENTS OF MATURE ENAMEL

Previous reviews have pointed out that there is a small amount of organic matter in mature dental enamel which is arranged in a very delicate fibrillar network visible only by electron microscopy (*Nutrition Reviews* **6,** *70* (*1948*); **11,** *54* (*1953*)). Histochemical studies of this organic matrix suggested the presence of traces of alkaline phosphatase, lipids and acid mucopolysaccharides as well as a type of keratin (*Ibid.* **9,** *268* (*1951*)). In addition, experiments with radioisotopes have shown that enamel is readily permeable to inorganic ions and that there is a slow rate of exchange between the inorganic elements of the enamel and those of the blood and saliva (*Ibid.* **11,** *89* (*1953*)). Although the exact pathways by which this permeability and exchange occurs are unknown, there is good reason to suspect that these natural phenomena are mediated to some extent by the organic components of the enamel.

In a recent paper, M. V. Stack (*J. Am. Dent. Assn.* **48,** *297* (*1954*)) has described a series of experiments in which the organic components of the enamel of human teeth have been studied in detail. The total organic content of the enamel from sound teeth was found to be an average of 0.60 per cent with a range of 0.56 to 0.65 per cent for the 10 samples studied. Analyses of the total organic content of sound enamel from partially decayed teeth gave comparable values. Other studies indicated that approximately 30 per cent of the organic matter, that is, 0.18 per cent of the original

enamel, was an insoluble protein with an amino acid ratio very similar to that of the eukeratins, which by definition are a class of insoluble proteins with a histidine-lysine-arginine ratio of 1:4:12. About 20 per cent of this insoluble protein dissolved when boiled with water for several hours. Amounts varying from 70 to 80 per cent of the insoluble protein dissolved when treated overnight at suitable pH levels with solutions of crystalline chymotrypsin, pepsin or trypsin, or with a collagenase preparation. It is interesting to note that carefully purified preparations of this insoluble protein still contained about 4.1 per cent of hydroxyproline, an amino acid which is characteristic of collagen and which is rarely found elsewhere.

In the soluble fraction of the organic matter from enamel, three types of compounds were detected: citric acid at a level of 0.10 per cent of the original enamel, soluble protein at 0.17 per cent and peptides at 0.15 per cent. The soluble protein contained 0.019 per cent glucose and 0.004 per cent hexosamine and was classed as a glycoprotein. As in the eukeratin, this soluble protein after purification also contained appreciable amounts of hydroxyproline.

In another publication, M. V. Stack (*Brit. Dent. J.* **96,** *73 (1954)*) has reported values for the organic content of enamel which has become chalky either in the earliest stages of tooth decay or as seen in areas surrounding or overlying active and extensive carious lesions. The former type of altered enamel contained 1.4 to 2.1 per cent organic matter in contrast to the 0.6 per cent of sound enamel. In this enamel surrounding or overlying active carious lesions, the organic content was 4.4 to 7.0 per cent. No data were presented to indicate which of the organic components had increased in either of these types of altered enamel. Possibly this increase in organic content was due to the presence of microorganisms in the altered enamel as well as to the leaching out of some of the inorganic salts.

These studies forcefully indicate the increasing rate at which fundamental data are being accumulated about dental enamel to broaden our understanding of its structure and metabolic potentialities. As recently as two decades ago, A. Hopewell-Smith (*New Zealand Dent. J.* **27,** *54 (1931)*) stated with conviction "everything indicates that the enamel is a solid, dense adamantive, inorganic mass." This was not by any means an isolated viewpoint, but one shared widely by prominent investigators in the dental field. Today newly published data from a variety of sources indicate not only that there is a structurally and chemically complex organic component of enamel, but also that there are metabolic potentialities therein. This is the type of knowledge which will enable a sound evaluation of the etiology of dental caries to be made and help in the formulation of procedures for the prevention of this disease.

VITAMIN B_{12} AND METHYL GROUP SYNTHESIS

The demonstration in du Vigneaud's laboratory that dietary methionine could be replaced by homocystine and an adequate methyl donor has become a keystone in the transmethylation story. It was observed, however, that occasionally an animal would show growth when given homocystine not supplemented with methyl donors. This has been attributed to the synthetic action of the intestinal flora. The tissue synthesis of methyl groups from such precursors as formate, methanol, serine, glycine and acetone is now also established. Various factors present in liver extract have been shown to influence methyl group synthesis. One of these factors is vitamin B_{12}. This

substance reduces the experimental animal's requirement for methyl donors when homocystine is fed in place of methionine. In its presence growth performance is improved, less kidney damage results and mortality is less. Vitamin B_{12} appears to exert no effect in the total absence of methyl donors. It seems to act by reducing the absolute dietary requirement for methyl groups.

H. R. V. Arnstein and A. Neuberger (*Biochem. J.* **55,** *259 (1953)*) have studied the relative efficiency of some methyl group precursors. Weanling rats were placed on a synthetic diet containing pure amino acids in place of protein. These rations were fed for at least twenty days and in many cases for a longer period. Such long-term feeding was resorted to by the authors in order to eliminate differences in reaction rate in the synthesis of methyl groups from precursors such as glycine, serine, or formate incorporated into the amino acid-containing diets. The amino acid mixture contained homocystine in place of methionine, in order to eliminate as completely as possible preformed, labile methyl groups. When this diet was supplemented with vitamin B_{12} but no choline, small daily weight gains were observed. When the diet contained suboptimal amounts of choline but no vitamin B_{12}, growth rates were minimal. Suboptimal choline supplementation, along with administration of vitamin B_{12}, resulted in moderate growth.

These experiments show clearly the beneficial effect of vitamin B_{12} when added to a ration containing suboptimal quantities of choline. Vitamin B_{12} supplementation was apparently more effective than choline supplementation alone. In these initial experiments, it was found that supplementation with both vitamin B_{12} and choline greatly increased the average daily food consumption. In another experiment the basal ration was supplemented with choline, some rats receiving vitamin B_{12} and being restricted in food intake to the amounts consumed by the control animals receiving no added vitamin B_{12}. Under these conditions vitamin B_{12} did not exert a choline-sparing action as judged by growth rates.

A study of the fate of formate carbon, the alpha-carbon of glycine and the beta-carbon of serine showed that there was greater incorporation of C^{14} from these sources into the methyl group of methionine than into the methyl group of choline. The authors speculate that the three precursors investigated here give rise first to the methyl groups of methionine which then secondarily find their way into choline by transmethylation. The incorporation of formate carbon into serine was shown to be much slower than its incorporation into the methyl groups of methionine. Consequently, the authors conclude tentatively that serine is not an intermediate in the pathway of transmethylation of formate into methyl groups.

In the absence of dietary vitamin B_{12}, methyl group synthesis still occurred. The authors acknowledge, however, that this may be due to stores of this vitamin laid down before restriction was imposed, or that intestinal synthesis of the factor continued throughout the experiment. Nevertheless, addition of vitamin B_{12} to the ration greatly increased the isotope content of methyl groups of methionine and choline, whether the precursor was glycine, serine, or formate. This effect was much reduced in the paired-feeding experiments, but was still present.

In a second paper of this series (*Biochem. J.* **55,** *271 (1953)*) H. R. V. Arnstein and A. Neuberger contribute some interesting quantitative information about the synthesis of glycine and serine. Their data suggest that glycine is derived largely from serine and that glycine synthesis occurs at a nearly constant rate, independent of the dietary level of glycine. The conversion of the alpha-carbon of glycine into the beta-carbon of serine was diminished when the dietary level of glycine was decreased. The authors

look upon this conversion as a means of disposing of excess dietary glycine.

In the third paper of the series, H. R. V. Arnstein and J. C. Crawhall (*Biochem. J.* **55,** *280 (1953)*) looked for the possible conversion of the beta-carbon of cysteine into methyl groups of methionine or choline. This was attempted because of the similarity of structure of serine and cysteine, as well as the resemblance of some aspects of their intermediary metabolism. Although the beta-carbon of serine is readily transformed into labile methyl groups, the corresponding beta-carbon of cysteine is not so converted.

The experiment showed secondarily that sulfur-labeled cysteine does not yield its sulfur to the synthesis of methionine via cystathionine. The major pathway of breakdown of cysteine appeared to be via pyruvate.

THE INFLUENCE OF UNDERNUTRITION ON EXPERIMENTAL ATHEROSCLEROSIS

The view that undernutrition in some way protects against experimental atherosclerosis has been challenged by the results of experiments by G. C. McMillan, J. H. Whiteside, and G. L. Duff (*J. Exp. Med.* **99,** *261 (1954)*). These workers have studied the severity of atherosclerosis in cholesterol-fed rabbits eating a natural diet and during undernutrition. Five different types of experiments were performed. In the first experiment young growing rabbits were fed 0.5 g. of cholesterol which was mixed with 35 g. of rabbit food and coated with vegetable oil. No other food was given to 15 rabbits whereas an additional ration of 65 g. of food without cholesterol was given to an equal number of rabbits. The diets were fed six days a week for eighty-one days. In addition to losing weight the undernourished rabbits had higher free and total serum cholesterol levels after the period of study than the adequately fed rabbits. The serum lipid phosphorus averaged 21.3 mg. per cent in the undernourished group and 15.3 mg. per cent in the control group. The amount and severity of the arteriosclerosis was equal in the two groups, judged by inspection.

In the second experiment the conditions were similar to the first except that each rabbit received 0.75 g. of cholesterol daily and the period of study was extended to ninety days. At the completion of the experiment only 6 of the 20 undernourished rabbits had survived and they weighed about 1 kg. less than control animals. The free and total serum cholesterol levels at the completion of the study were 683 and 1914 mg. per cent respectively in the undernourished group as compared to 175 and 651 mg. per cent in the control group. The serum lipid phosphorus was more than twice as high in the undernourished rabbits. The same degree of arteriosclerosis was found in both groups as judged by inspection. However, the cholesterol content of the aorta of the undernourished group was almost twice that of the control group.

In the next experiment the effect of a period of undernutrition before cholesterol feeding was studied. For a period of sixty days one of a pair of littermates received 35 g. of food while his mate received 100 g. of food. While continued on the same basal diet, cholesterol, 0.75 g., and vegetable oil, 6 g., were fed for an additional one hundred and ten days. One half of the pairs of rabbits survived this experiment. The serum cholesterol after the first sixty days and before cholesterol feeding was 15 mg. per cent higher in the undernourished rabbits as compared to their controls. After cholesterol feeding the serum levels were again higher in the undernourished group. In 5 of 10 pairs of rabbits the degree of atherosclerosis was the same in both the undernourished and control rabbit. In 3 pairs the undernourished rabbits had less atherosclerosis.

Two pairs were excluded from consideration because of atypical findings which were difficult to interpret.

In the fourth experiment the effect of feeding cholesterol to fully grown rabbits was observed. For fifteen weeks 20 rabbits were fed 35 g. of rabbit food containing 0.5 g. of cholesterol in vegetable oil. The control rabbits received an additional ration of 65 g. of rabbit food. The average content of cholesterol and lipid phosphorus in the serum was again found to be higher in the undernourished animals. The atherosclerotic lesions in both groups of rabbits were comparable.

In the last experiment the effect of undernutrition on the regression of fully developed aortic lesions was studied. Atherosclerotic lesions were presumably induced by cholesterol feeding for a period of eight weeks in a group of 40 young rabbits. All the animals were then allowed to eat an adequate diet without additional cholesterol for two weeks to allow the hypercholesterolemia to subside. The animals were then divided into an undernourished group (food intake 25 to 35 g. a day) and a control group (food intake 100 g. a day). It was noted that the serum cholesterol remained higher in the undernourished rabbits than in the control group. The aortas of this group did not show an acceleration of regression of the experimental atherosclerosis.

In these carefully recorded experiments the effect of undernutrition on the atherosclerosis of cholesterol-fed rabbits has been examined in a number of ways. In every case it has been found that undernutrition was without significant effect on the development or the regression of aortic lesions. The serum cholesterol, both free and combined, was distinctly higher in the undernourished animals. In only one respect might the feeding of a calorically inadequate diet be considered protective against atherosclerosis. The atherosclerosis of the undernourished rabbits was no worse than that of the control rabbits despite higher cholesterol levels.

The application of these experiments to human atherosclerosis is difficult, and caution is needed in their interpretation. There is some clinical evidence that in man atherosclerosis is accelerated in obesity and decreased under conditions of caloric insufficiency. There are certain obvious differences in the experimental and clinical situation. In the rabbit experiments of McMillan *et al.*, fat and cholesterol intake were maintained at a high level in both the undernourished and control groups. Human subjects on calorically inadequate diets often have low intakes of these substances.

THE SYNTHESIS OF MILK FAT

A good deal is known already about the chemical mechanisms involved in the synthesis of body fat from dietary carbohydrate and from protein. Similarly, we have much information about the transformations which food fats must undergo before they can be stored as fats characteristic of the animal and the organ in which they are to be deposited. We know that acetate is probably the chief starting material from which new fat is formed and that the acetate arises chiefly from carbohydrate and to some extent from certain amino acids. Many experiments now point to the necessity of burning some carbohydrate to furnish the energy for the transformation of acetate into the fatty acid portion of fats. Furthermore, the modulating role of hormones such as insulin, as well as some from the anterior pituitary and the adrenal glands, in fat synthesis is slowly being clarified. There has, however, been relatively little work on the precise chemical mechanisms by which the mammary gland synthesizes milk fat.

Investigations of this sort often pass through several common and clearly defined

stages. First comes the work with intact animals, modifying experimental conditions to test their influence on the processes under investigation. The next step, quite often, involves the use of very thin slices of some living tissue. Many chemical substances penetrate readily into the cells comprising the tissue slice and are there transformed in much the same fashion as in the whole animal. This technic can give relatively precise information because the products of the transformation can be detected, and the conditions necessary for the reaction can be ascertained.

A further refinement is the use of extracts of tissues. These preparations, if successful, contain all the cell components necessary for a given transformation but are free of cells. Their use avoids some of the difficulties encountered with tissue slices. The next step in the solution of problems in chemical transformation is the isolation of the pure enzymes involved in the reactions, along with the identification of the necessary co-factors.

The problem of milk fat synthesis by the mammary gland has now been pursued to the point where cell-free extracts, capable of converting acetate into fatty acids, have been obtained. This has been reported by G. Popják and A. Tietz (*Biochem. J.* **56,** *46 (1954)*). The tissues, taken largely from lactating animals, were repeatedly homogenized and then centrifuged at low temperatures to remove cell fragments and nuclei. No intact cells or cell nuclei could be demonstrated in these preparations. The cell-free suspensions along with suitable substrates and co-factors were incubated at 37.5°C.

Some preliminary experiments were carried out on the utilization of acetate for fatty acid synthesis by slices of sheep udder. It was found that these tissue slices were capable of converting acetate into fatty acid without the addition of glucose to the medium. The sugar, on the other hand, did stimulate fatty acid synthesis. Slices of rat mammary gland, however, did not utilize acetate for the synthesis of fatty acid in the absence of a co-substrate such as glucose. To some extent pyruvate, oxaloacetate,[1] alpha-ketoglutarate, and succinate could replace glucose as co-substrate. These compounds are all intermediates formed momentarily during the oxidation of glucose to carbon dioxide and water. These observations indicate that oxidation of glucose must be taking place to attain maximal rates of fat synthesis in mammary glands.

Cell-free preparations of rat mammary gland behaved very much like slices of this same tissue. In the absence of a co-substrate they were capable of incorporating only minute amounts of acetate into fatty acid. Unlike tissue slices, glucose or glucose with ATP (adenosine triphosphate) did not stimulate fatty acid synthesis in cell-free preparations. The highest rate of synthesis was noted in the presence of oxaloacetate and ATP. In the absence of ATP the cell-free preparations converted only small quantities of acetate into fatty acid when oxaloacetate was used as co-substrate. On the other hand, alpha-ketoglutarate greatly stimulated fatty acid synthesis even in the absence of added ATP. Adenosine triphosphate is a chemical substance whose stored energy is used universally to furnish energy for work processes such as the synthesis of large molecules from small ones.

The individual fatty acids synthesized by cell-free preparations of rat mammary gland were separated by chromatography. The cell-free suspension had been incubated with acetate, oxaloacetate and ATP. Ninety-one per cent of the newly synthesized fatty acid could be accounted for by caproic (6 carbons), myristic (14 carbons), palmitic (16 carbons), and oleic (18 carbon, unsaturated) acids. Myristic, palmitic and oleic acids comprise more than 80 per cent of the fatty acids found in milk.

NOTES

Strepogenin Activity of Synthetic Oxytocin

A new growth factor required by certain hemolytic streptococci was reported by D. W. Woolley several years ago (*J. Exp. Med.* **73,** *487 (1941)*). Subsequent work has shown that the factor, which was designated strepogenin, is formed during the partial hydrolysis of certain proteins. Woolley and R. B. Merrifield (*J. Am. Chem. Soc.* **76,** *316 (1954)*) have separated from the partial hydrolysis products of insulin a peptide with high strepogenin activity. It was noted that the amino acid composition of this peptide resembled that of the pituitary hormone oxytocin as reported by V. du Vigneaud *et al.* (*Ibid.* **75,** *4879 (1953)*). Woolley and Merrifield (*loc. cit.*) determined the strepogenin potency of synthetic oxytocin and found it to be very active. Slight changes in the molecule greatly reduced or abolished its strepogenin activity. However, other peptides, notably those obtained by partial hydrolysis of insulin, also possess high strepogenin activity; hence this activity does not reside in a single peptide structure. The fact that synthetic oxytocin was found to possess high strepogenin activity indicates that the activity of peptides obtained from natural proteins actually is due to the peptide structure and not to some contaminant.

Protein Supplementation of Wheat Flour and Corn Meal by Polished Rice

The world's food supply is dominated by the more economical cereal grains as sources of both protein and carbohydrate. Rice has been the food of the yellow and brown races while wheat has been the preference of the white race. Proteins of milled rice are superior biologically to those of milled wheat and corn. The possible supplementary value of the proteins of polished rice added to those of milled wheat flour and milled white corn meal has been studied by B. Sure, L. Easterling, J. Dowell, and M. Crudup (*J. Agr. Food Chem.* **1,** *1207 (1953)*), who conducted growth experiments on rats during an eight-week experimental period. The cereal products were the only sources of protein in the ration and were fed to supply the necessary protein levels.

When one half of the proteins in milled wheat was replaced by an equivalent amount of protein in milled rice, there were increases of 114 per cent in growth and 86 per cent in protein efficiency ratio; when one-third replacement was made by rice protein, there was a 55 per cent increase in growth and a 57 per cent increase in the protein efficiency ratio.

The proteins of polished rice showed much greater supplementary value over those of milled corn meal than over the proteins of milled wheat. When one-half replacement by rice proteins was used there were increases of 236 per cent in body weight and 165 per cent in protein efficiency ratio; when one-third replacement was in effect, the increases were still 190 and 148 per cent, respectively. These effects were interpreted as due to the proteins and amino acids in milled rice and not to other components of this product.

Thus rice, which enters into the white man's diet to such a minor extent, might contribute greatly to his protein nutrition, particularly in the South where corn meal is the basic breadstuff. For people of low income levels who use mostly corn for their basic cereal foods, those in the southern part of the United States, Guatemala, South America, Yugoslavia and Mexico, it would seem desirable to consume a greater proportion of rice to balance the proteins of corn, which are known to be deficient in lysine, tryptophan, threonine and methionine.

CLINICAL NUTRITION BRIEFS

"Patients suffering from nutritional edema were found to have a slightly low serum sodium (mean 136.3 mEq per liter), a low serum chloride (mean 87.6 mEq per liter), and a slightly elevated serum potassium level (5.2 mEq per liter). Following diuresis induced by a high protein diet the serum sodium actually showed a further decrease (mean 131.7 mEq per liter), indicating that sodium had been lost at an inappropriate rate as compared to water. The levels of serum chloride and potassium in the non-edematous stage had returned toward normal." (*Nutrition Reviews* **12,** *100* (*1954*), Body Composition in Nutritional Edema.)

"The weight of the fetal liver bears a closer relationship to the protein intake of the mother than to her over-all dietary intake. No correlation was found between vitamin A content in the fetal liver and carotene content of the maternal diet. However, the content in the fetal liver was adversely affected by a poor maternal intake of vitamin A. Mothers who took mineral oil regularly during pregnancy also tended to produce fetuses whose livers contained less than a normal amount of vitamin A." (*Nutrition Reviews* **12,** *104* (*1954*), Maternal Diet and Fetal Liver.)

"It is possible to summarize the requirements for parenteral protein repletion: (1) a source of amino acid nitrogen containing the proper mixture of essential amino acids, (2) the simultaneous administration of adequate calories in a readily utilizable form—usually carbohydrate, (3) provision of a minimum of 4 mEq of potassium for each gram of amino acid nitrogen." (*Nutrition Reviews* **12,** *107* (*1954*), Potassium and Parenteral Protein Alimentation.)

"These observations demonstrate that the patient with pernicious anemia is able to absorb vitamin B_{12} from the intestinal tract when large quantities of the vitamin are administered. Moreover, the serum concentration of the vitamin increases in these patients as it does in normal subjects given similar quantities by injection or by mouth. These findings are consistent with the clinical observations that hematologic response may be induced and maintained in patients with pernicious anemia when large quantities of vitamin B_{12} are administered by mouth." (*Nutrition Reviews* **12,** *105* (*1954*), Serum Concentration and Urinary Excretion of Vitamin B_{12}.)

"Stable diabetic subjects responded to glucagon and to epinephrine in a manner roughly similar to that of the normal subjects. By contrast, unstable diabetic subjects failed to show an appreciable rise in blood glucose after glucagon administration, although they demonstrated a striking increase in blood glucose after epinephrine administration." (*Nutrition Reviews* **12,** *132* (*1954*), Glucagon.)

"The metabolic study of these patients during recovery from beriberi heart disease indicated movement of sodium, potassium and water between intracellular and extracellular fluid compartments so that there was increase in the total electrolyte in cells." (*Nutrition Reviews* **12,** *162* (*1954*), Distribution of Body Fluid and Electrolytes in Heart Failure Due to Beriberi.)

"At an age of from 37 to 39 weeks infants receiving ferrous iron exhibited an average hemoglobin level of 13.1 g. per cent (standard error 0.31) as compared to 11.7 g. per cent (standard error 0.20) for the group receiving ferric iron. Thus at the dosage employed ferrous iron supported a higher hemoglobin level for infants than did ferric iron." (*Nutrition Reviews* **12,** *192* (*1954*), Ferric Versus Ferrous Iron for Prevention of Hypochromic Anemia in Infants.)